PATENT
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	
Michael Eppihimer, et al.	Group Art Unit: 1644
Serial No.: 09/825,580	Examiner: GAMBEL, P.
Filed: April 2, 2001	Confirmation No.: 9952
For: INHIBITION OF THROMBOSIS BY TREATMENT WITH P-SELECTIN ANTAGONISTS	

Attention: Mail Stop Appeal Brief-Patents
Commissioner for Patents
P.O. Box 1450
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Sir:

APPEAL BRIEF UNDER BOARD RULE § 41.37

In support of the Notice of Appeal filed December 28, 2006, and further to Board Rule 41.37, Appellant presents this brief and enclose herewith a check for the fee of $500.00 required under 37 C.F.R. § 1.17(c).

This Appeal responds to the September 28, 2006, rejection of Claims 1-20, 25-27, 31-40, and 45, and 50-57, which are set forth in the attached Appendix.

If any additional fees are required or if the enclosed payment is insufficient, Appellant requests that the required fees be charged to Deposit Account No. 06-0916.

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I. Real Party In Interest

Genetics Institute, L.L.C. is assignee of record as evidenced by the assignment recorded on March 29, 2002, at reel 12772, frame 631, and as such, is the real party in interest in this appeal. Genetics Institute, L.L.C. is a subsidiary of Wyeth.

II. Related Appeals and Interferences

There are currently no other appeals or interferences, of which appellant, appellant's legal representative, or assignee are aware, that will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

III. Status Of Claims

Claims 1-20, 25-27, 31-40, and 45, and 50-57 are pending in this application and are currently rejected. Claims 21-24, 28, 41, and 42 are cancelled, while claims 29, 30, 43, 44, and 46-49 are withdrawn. The claims are provided in an Appendix to the appeal brief. As argued below, Appellants believe that the rejected claims are patentable.

IV. Status Of Amendments

The most recent amendments were made on September 13, 2006. Therefore, all amendments to the specification and claims have been entered, and no amendments have been made subsequent to the September 13, 2006, Response.

V. Summary Of Claimed Subject Matter

The present invention relates to methods of treating thromboses in a subject having hypertension. In particular, the method involves providing a patient a P-selectin ligand glycoprotein ligand 1 (PSGL-1). PSGL-1 is a high affinity ligand for P-selectin, and it may also bind to E-selectin and L-selectin. *Specification*, page 2, lines 6-7. PSGL-1 is expressed by leukocytes and mediates cell adhesion between leukocytes, platelets and endothelial cells. *Id.* at 8-9. Cell adhesion, in turn, plays a role in thrombosis, which is the formation of a blood clot or thrombus. *Id.* at 1, line 5. Thromboses may form following blood vessel injury by invasive procedures such as angioplasty or coronary bypass surgery, or may be caused by cardiovascular conditions. *Id.* at lines 23-32. Thrombosis is a serious medical condition that can cause tissue damage and, if untreated, death. *Id.* at 1, lines 18-19.

The present invention is based, in part, on the discovery that antagonists of P-selectin, including soluble PSGL-1 protein and PSGL fusion protein, inhibit cellular adhesion, thereby inhibiting formation of thrombosis. Thus, providing a patient with a PSGL-1 protein could treat or inhibit thrombus formation. The current claims focus on treatment of a subject having hypertension. While subjects with a variety of different conditions could be treated with the claimed method, Appellants selected "hypertension" at the request of the Examiner. *See* Interview Summary attached to Office Action mailed September 9, 2004.

Independent claim 1 focuses on a method of treating or inhibiting thrombosis in a subject suffering from hypertension. It recites a method comprising administering to a

subject having hypertension a composition comprising a PSGL-1 protein which has P-selectin ligand activity. The recited P-selectin ligand activities include a) inhibiting P-selectin or E-selectin binding; b) inhibiting adhesion of at least one of leukocytes to endothelial cells, leukocytes to platelets, leukocytes to blood vessels, and platelets to blood vessels; c) inhibiting leukocyte recruitment to platelets and endothelial cells; d) increasing leukocyte migration; e) increasing movement of at least one of leukocytes and platelets relative to blood vessels; and f) increasing leukocyte rolling velocity.

Independent claim 25 focuses on a method of inhibiting thrombus that is induced by a thrombus-inducing agent in a subject having hypertension. It recites a method comprising identifying a subject having hypertension and administering to the subject a composition comprising an effective amount of soluble PSGL-1 protein or a fragment thereof having a P-selectin ligand activity chosen from the same activities that are recited in independent claim 1.

Independent claim 31 is directed to a method of preventing deep vein thrombosis (DVT). DVT is the formation of thrombus within a deep vein. Specification, p. 1, lines 8-9. Claim 31 recites a method comprising identifying a subject having or at risk for DVT and administering to the subject a composition comprising an effective amount of a soluble PSGL-1 protein or a fragment thereof having a P-selectin ligand activity chosen from the same activities that are recited in independent claim 1.

Independent claim 45 focuses on a prophylactic method of treating or inhibiting thrombosis in a subject with hypertension. It recites a method comprising identifying a

subject at risk of thrombosis due to hypertension and administering to the subject a composition comprising an effective amount of a PSGL-1 protein or a fragment thereof having a P-selectin ligand activity chosen from the same activities that are recited in independent claim 1.

Independent claim 57 is directed to a method of treating, inhibiting or preventing thrombosis in a subject at risk for thrombosis. It recites a method comprising identifying a human subject at risk of thrombosis due to hypertension and administering to the subject a composition comprising an effective amount of a soluble PSGL-1 protein or fragment thereof having a P-selectin activity chosen from the same activities that are recited in independent claim 1.

VI. Grounds of Rejection

A. Claims 1-4, 8-13, 16-18, 25-27, 45-47, 50-53, and 57 stand rejected under 35 U.S.C. § 102(e).

B. Claims 1-20, 25-27, 31-40, 45, and 50-57 stand rejected under 35 U.S.C. § 103(a).

VII. Argument

A. The Subject Matter Of The Claims Is Not Present Literally Or Inherently In The Prior Art

In the Office Action mailed September 28, 2006, the Office rejects claims 1-4, 8-13, 16-18, 25-27, 45-47, and 50-53 under 35 U.S.C. § 102(e) as inherently anticipated by U.S. Patent No. 5,464,778 ("Cummings") as evidenced by THE MERCK MANUAL OF DIAGNOSIS AND THERAPY 1655 (17th ed. 1999) ("Merck Manual") and Lip *et al.*, "Hypertension and the prothrombotic state," J. Hum. Hyper. 14: 687-90 (2000) ("Lip"). Office Action, p. 3.

Appellants respectfully assert that claims 1-4, 8-13, 16-18, 25-27, 45-47, 50-53, and 57 are not anticipated by the prior art because these claims recite subject matter that is not present literally or inherently in the prior art. In rejecting these claims, the Office relies on an improper standard for finding inherent anticipation. The prior art relied on by the Office does not disclose that hypertension is necessarily present in patients with thrombosis. Appellants also rely on a Declaration of Dr. Stefan Hemmerich on September 13, 2006. This Declaration is included in the attached Appendix.

1. A Finding Of Inherent Anticipation Requires That The Missing Descriptive Matter Is Necessarily Present In The Applied References

A claim is anticipated "only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." MPEP § 2131 (8th ed., 2d rev. 2004) (citing *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631 (Fed. Cir. 1987)). Normally, only a single reference should be used in

rejecting an application under 35 U.S.C. § 102, though a § 102 rejection over multiple references has been found proper where the additional reference was cited: (1) to prove the primary reference contains an enabled disclosure; (2) to explain the meaning of a term used in the primary reference; or (3) to show that a characteristic not disclosed in the primary reference is inherent. MPEP § 2131.01. The reference "must make clear that the missing descriptive matter is necessarily present in the thing described in the reference." MPEP § 2112 (citing *Schering Corp. v. Geneva Pharmaceuticals, Inc.*, 339 F.3d 1373, 1376 (Fed. Cir. 2003)) (emphasis added). Finally, inherency "may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient." MPEP § 2112 (citing *In re Robertson*, 169 F.3d 743, 745 (Fed. Cir. 1999)). The burden is on the Office to "provide a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent characteristic necessarily flows from the teachings of the applied prior art." MPEP § 2112 (citing *Ex parte Levy*, 17 U.S.P.Q.2d 1461, 1464 (Bd. Pat. App. & Inter. 1990)).

The recent Federal Circuit decision of *Perricone v. Medicis*, 432 F.3d 1368 (Fed. Cir. 2005), in which the court found lack of inherent anticipation, is instructive. In *Perricone*, the court considered whether a claim to a method of treating sunburn using a particular formulation was inherently anticipated by a prior patent that disclosed a similar formulation for use on skin. As stated by the court, "[i]f Pereira [the prior art] discloses the very same methods, then the particular benefits must naturally flow from those methods, even if not recognized as benefits at the time of Pereira's disclosure."

Perricone F.3d at 1378 (emphasis added). In finding that the claimed method was not inherently anticipated, the court stated "[t]he issue is not, as the dissent and the district court imply, whether Pereira's lotion would inherently treat that damage, but whether Pereira discloses the application of its composition to skin sunburn." *Id.* at 1378. The court concluded "[i]t does not," and that the claimed method of treating sunburn "recites a new use of the composition disclosed by Pereira, the treatment of sunburn." *Id.* at 1337-79. Thus, the Federal Circuit clearly indicates that the mere possibility that a compound disclosed in the prior art could function in a particular manner does not preclude the patenting of a new use for the compound.

The Federal Circuit's decision in *Schering Corp. v. Geneva Pharmaceuticals, Inc.*, 339 F.3d 1373, 1376 (Fed. Cir. 2003) demonstrates the close relationship that must exist between the prior art and the later claimed invention for a finding of inherent anticipation. In *Schering*, the plaintiff obtained a patent that claimed the antihistamine loratadine, and obtained a later patent that claimed a metabolite of loratadine, DCL. The district court granted summary judgment to defendants who argued that the disclosure of loratadine inherently anticipated its metabolite, DCL. *Schering*, 339 F.3d at 1374. The Federal Circuit affirmed the lower court's ruling. *Id.* at 1382. The Federal Circuit noted that the metabolite was not expressly disclosed in the antihistamine patent, but that "the record demonstrated that DCL necessarily and inevitably forms from loratadine under normal conditions. DCL is a necessary consequence of administering loratadine to patients". *Id.* at 1378 (emphasis added). Accordingly, a loose association between the disclosure of the alleged anticipatory reference and the

later claimed subject matter is not sufficient to find anticipation. Rather, there must be a necessary relationship between the two.

2. Hypertension Is Not Necessarily Associated With The Conditions Of Cummings

In rejecting claims 1-4, 8-13, 16-18, 25-27, 45-47, 50-53, and 57, the Office fails to meet its burden in showing that hypertension is necessarily associated with the conditions of Cummings. The Office does not argue that the claimed invention is explicitly disclosed in the prior art, but cites The Merck Manual and Lip to show that hypertension is inherent in the conditions of Cummings. Office Action, p. 3. Cummings discusses the treatment of several conditions including atherosclerosis, stroke, and conditions produced by ischemia/reperfusion injury. *See* col. 18, line 54 to col. 19, line 20, and col. 19, line 64 to col. 20, line 5. Cummings does not teach that these conditions are associated with hypertension.

The reliance on The Merck Manual by the Office is misplaced because The Merck Manual actually exemplifies the distinct nature of hypertension and the conditions of Cummings. For example, the Merck Manual describes the characteristics of atherosclerotic vessels and then describes the distinct characteristics of such vessels when hypertension is present. THE MERCK MANUAL OF DIAGNOSIS AND THERAPY 1655 (17th ed. 1999). This description indicates that hypertension and atherosclerosis need not coexist. In fact, hypertension is not listed as a symptom characteristic of atherosclerosis in the passage of the Merck Manual cited by the Office, which states that "[a]therosclerosis is characteristically silent until critical stenosis,

thrombosis, aneurysm, or embolus supervenes." THE MERCK MANUAL OF DIAGNOSIS AND THERAPY 1657 (17th ed. 1999).

Moreover, the Office cites passages of the Merck Manual that describe hypertension as a "risk factor" for several diseases. Office Action, p. 3. For example, the Office quotes the Merck Manual's statement that hypertension is one of "three risk factors, along with cigarette smoking and hypercholesterolemia predisposing to coronary atherosclerosis" and hypertension is the "most important risk factor predisposing to stroke." *Id.* The Merck Manual's treatment of hypertension as one of several "risk factors" highlights how certain conditions may alter the probability or possibility of a particular disease but that such factors are not necessarily associated with the disease. A risk factor may be more "important" than others, suggesting a stronger association between the disease and the risk factor. Nevertheless, the risk factor indicates a probability or possibility of association, not a necessary association.

Lip also fails to demonstrate that hypertension is necessarily associated with the conditions of Cummings. Similar to The Merck Manual, Lip describes the association as one of risk, not certainty. Lip describes haemostatic abnormalities that "appear to be additive to conventional risk factors for cardiovascular and cerebrovascular events." Lip, p. 687 (emphasis added). If fact, far from teaching a necessary association between hypertension and the conditions of Cummings, Lip discusses the uncertain relationship between the two. Lip speculates that, "[s]ince the processes of thrombogenesis and atherogenesis have certain similarities to inflammatory disease, the elevations in various indices may reflect the severity of vascular disorders as a secondary phenomenon rather than act as a true prognostic factor." *Id.* at 689. Thus,

Lip makes clear that there is not a necessary association between hypertension and cardiovascular conditions, and Lip further indicates that there was uncertainty about the significance of any correlation between these phenomena when the invention was made. Accordingly, The Merck Manual and Lip do not support the contention that hypertension is necessarily associated with the conditions of Cummings, and Cummings does not inherently anticipate the claimed method.

3. It Is Known To Those Of Ordinary Skill In The Art That Hypertension Is Not Necessarily Present In The Conditions Of Cummings

The Merck Manual indicates that there may be a correlation between hypertension and various conditions, but hypertension is just one of many risk factors that might predispose a patient to certain conditions. It is well known by those skilled in the art that patients with atherosclerosis need not also have hypertension. See Hemmerich Declaration, paragraph 7(A).

The Merck Manual teaches that strokes can be caused by arteriosclerotic or hypertensive stenosis, thrombosis or embolism. (See page 1421). The Merck Manual does not teach that stroke is necessarily associated with hypertension. In totality, the Merck Manual indicates that hypertension and stroke do not always coexist and patients suffering from a stroke do not always have hypertension. This is well known among those skilled in the art. See Hemmerich Declaration, paragraph 7(B).

The Merck Manual teaches that a number of factors including hypertension predispose a patient to Transient Ischemic Attacks (TIA). However, it is known to those

of skill in the art that ischemia and hypertension need not always coexist. See Hemmerich Declaration, paragraph 7(C).

The Office has not shown inherent anticipation by Cummings. Cummings does not teach that hypertension is necessarily associated with the conditions of Cummings, and neither The Merck Manual not Lip show that hypertension is necessarily present in the diseases of Cummings. Moreover, those of skill in the art recognize that hypertension is not necessarily present in the diseases in Cummings. *See* Hemmerich Declaration. Accordingly, the instantly claimed invention is not inherently anticipated, and Appellants respectfully request that the rejection of claims 1-4, 8-13, 16-18, 25-27, 45-47, 50-53, and 57 be withdrawn.

B. The Subject Matter Of The Claims Is Not Obvious

The Office maintains the prior rejection of claims 1-20, 25-27, 31-40, 45, and 50-57 under 35 U.S.C.§ 103(a) as allegedly unpatentable over Cummings and Larsen *et al.*, U.S. Patent No. 5,840,679 ("Larsen") in view of Blann *et al.*, "Evidence of platelet activation in hypertension," J. Hum. Hyper. 11:607-609 (1997) ("Blann"), Araneo *et al.*, U.S. Patent No. 6,150,348 ("Araneo") and DeFrees *et al.*, U.S. Patent No. 5,604,207 ("DeFrees"), and further in view of the Merck Manual. Office Action, p. 5. The Office apparently contends that administration of PSGL-1 to treat the conditions recited in Cummings and Larsen would inherently treat hypertension, and therefore thrombosis. *Id.*

Appellants respectfully assert that the claimed invention is not obvious in view of the publications cited by the Office. Hypertension and thromboses need not coexist.

Accordingly, the skilled artisan would not know if a patient suffering from hypertension also suffered from thromboses, and it would not be obvious to treat such a subject with PSGL-1. A described in detail below, none of the publications relied upon by the Office supply the necessary link between hypertension, thromboses and treatment with PSGL-1. As such, these publications, whether considered alone or together, fail to make out a *prima facie* case of obviousness.

A proper *prima facie* obviousness rejection requires some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. *See* M.P.E.P. § 2143. The Examiner bears the burden of establishing *prima facie* obviousness. *See* M.P.E.P. § 2142.

1. There is No Motivation to Combine References

In rejection the claimed methods as obvious, the relies upon an improper finding of inherency, and thus motivation to combine references is lacking. Cummings and Larsen neither teach nor suggest the use of a PSGL-1 protein for treating or inhibiting thrombosis in a patient with hypertension. As noted above, Cummings neither teaches nor suggests that hypertension is necessarily associated with any of the conditions discussed therein, and the lack of necessary association between hypertension and the conditions of Cummings was known to those of skill in the art. *See* Hemmerich Declaration, paragraph 8. Larson, Blann, Araneo, Defrees and The Merck Manual also fail to show such a necessary association and do not provide the motivation to arrive at the claimed invention.

a. Cummings

As noted above, Cummings discusses the treatment of several conditions including atherosclerosis, stroke, and conditions produced by ischemia/reperfusion injury. *See* col. 18, line 54 to col. 19, line 20, and col. 19 line 64 to col. 20, line 5. Cummings does not teach that these conditions are associated with hypertension.

b. Larsen

Larsen describes a P-selectin ligand protein, and methods of treating numerous conditions using P-selectin ligand (See column 15, lines 50-66). Larsen does not mention treatment of subjects with hypertension nor does Larsen teach that conditions that might be treated with P-selectin ligand are associated with hypertension. Larsen also fails to teach that a composition having P-selectin ligand activity could be used to treat or inhibit thrombosis, thrombus formation or deep vein thrombosis in a subject having hypertension. The disclosure in Larsen, whether alone or when combined with Cummings, would not suggest to the skilled artisan that a composition having P-selectin ligand activity could be used to treat or inhibit thrombosis, thrombus formation or deep vein thrombosis in a subject having hypertension. *See* Hemmerich Declaration, paragraph 10.

c. Blann

Blann speculates that compounds that reduce platelet activity, such as aspirin, could be useful to treat thrombosis but does not teach that a composition having P-selectin ligand activity could be used to treat or inhibit thrombosis, thrombus formation, or deep vein thrombosis in a subject having hypertension. (Blann, page 608). There is no suggestion in Blann that PSGL-1 could be substituted for the compounds discussed

in Blann, and Blann provides no motivation to do so. Moreover, to one skilled in the art, the disclosure in Blann would not suggest that a composition having P-selectin ligand activity could be used to treat or inhibit thrombosis, thrombus formation or deep vein thrombosis in a subject having hypertension. *See* Hemmerich Declaration, paragraph 11.

d. Araneo

Araneo discusses methods of preventing or reducing the effects of ischemia and other conditions including pulmonary hypertension by administering the steroid DHEA, a very different compound from the instantly claimed protein. (*See* Abstract. *Also see* column 4). Araneo does not teach or suggest that a composition having P-selectin ligand activity could be used to treat or inhibit thrombosis, thrombus formation, or deep vein thrombosis in a subject having hypertension, but suggests a treatment based on reducing the level of P-selectin expression. (*See* column 17, lines 59-64). Araneo does not teach or suggest methods of treatment of a subject suffering from hypertension with PSGL-1. To one skilled in the art, the disclosure in Blann would not suggest that a composition having P-selectin ligand activity could be used to treat or inhibit thrombosis, thrombus formation or deep vein thrombosis in a subject having hypertension. *See* Hemmerich Declaration, paragraph 12.

e. DeFrees

DeFrees describes analogs of sialyl Le^x and speculates about the use of these compounds to treat inflammatory disorders, and mentions the use of these analogs to treat deep vein thrombosis. (*See* column 3 and column 44, lines 35-65; *see also* column 45, lines 7-15). However, DeFrees does not teach or suggest to the skilled

artisan the treatment of deep vein thrombosis in a subject with hypertension. DeFrees fails to even mention hypertension. To the skilled artisan, DeFrees fails to suggest any relationship between P-selectin or PSGL-1 and the treatment of thrombosis in a subject with hypertension. *See* Hemmerich Declaration, paragraph 13.

f. The Merck Manual

As noted above, The Merck Manual merely describes a correlation between hypertension and certain conditions, but hypertension is just one of many risk factors that might predispose a patient to these conditions. Moreover, it is well known by the skilled artisan that patients with thrombotic conditions need not also have hypertension. *See* Hemmerich Declaration, paragraphs 7-8.

Because of the shortcomings of Blann, Araneo, DeFrees and the Merck Manual, these publications fail to cure the deficiencies of Cummings and Larsen. First, none of these references teach or suggest that hypertension is necessarily associated with the conditions discussed in Cummings and/or Larsen, and it would not be obvious to treat a patient suffering from a condition of Cummings and/or Larsen as the skilled artisan would not know whether the patient had hypertension. Second, each of Blann (aspirin), Araneo (hormone), and DeFrees (analogues of sialyl-Lewis^x) discuss compounds other than a PSGL-1 protein. In the absence of a known or inherent association between hypertension and the conditions of Cummings or Larsen, and a teaching or suggestion to substitute PSGL-1 for the variety of compounds disclosed, one of skill in the art would have no motivation to arrive at the claimed invention by combining references.

2. Claim 27 is Not *Prima Facie* Obvious

The Office maintains the prior rejection of claim 27 under 35 U.S.C.§ 103(a) as allegedly unpatentable over Cummings and Larsen, in view of Blann, Araneo, DeFrees, the Merck Manual, as applied to claims 1-20, 25-27, 31-40, 45, and 50-57 above, and further in view of Maugeri *et al.*, "Polymorphonuclear Leukocyte-Platelet Interaction: Role of P-selectin in Thromboxane B_2 and Leukotriene C_4 Cooperative Synthesis," *Thromb. Haem.* 72:450-456 (1994) ("Maugeri") and Johnston *et al.*, "Differential Roles of Selectins and the α4-Integrin in Acute, Subacute, and Chronic Leukocyte Recruitment In Vivo," *J. Immunol.* 159:4514-4523 (1997) ("Johnston"). Office Action, p. 10. The Office concedes that Cummings and Larsen do not disclose the role of LTC_4 in thrombus formation or thrombotic conditions *per se*, but maintains that LTC_4 was a known thrombus-inducing agent involved in thrombus formation and thrombotic conditions, as allegedly shown by Maugeri and Johnston. *Id.* at 11.

a. Maugeri

Maugeri investigates a relationship between LTC_4 and the aggregation of mixtures containing platelets and polymorphonuclear leukocytes, and describes decreased aggregation of these mixtures in the presence of an anti-P-selectin antibody *in vitro*. (See Introduction and Figure 2). Maugeri does not mention the use of a P-selectin ligand protein to treat thrombosis, and does not mention any relationship between thrombosis formation and hypertension. To one of ordinary skill in the art, the disclosure of Maurgeri would not suggest a method of treating or inhibiting thrombus formation induced by a thrombus-inducing agent in a subject comprising identifying a subject having hypertension and administering to the subject a composition of a PSGL-

1 protein, or said method wherein the thrombus inducing agent is LTC_4. See Hemmerich Declaration, paragraph 15.

b. Johnston

Johnston investigates the ability of anti-P-selectin antibodies to inhibit LTC_4-induced leukocyte rolling *in vitro* (See, e.g., Figure 1). Johnston speculates about anti-inflammatory strategies designed to block leukocyte recruitment but does not identify the use of a P-selectin protein. (See page 4532). Moreover, Johnston fails to teach or suggest any relationship between thrombus formation and hypertension. To one of ordinary skill in the art, the disclosure of Johnston would not suggest a method of treating or inhibiting thrombus formation induced by a thrombus-inducing agent in a subject comprising identifying a subject having hypertension and administering to the subject a composition of a PSGL-1 protein, or said method wherein the thrombus inducing agent is LTC_4. *See* Hemmerich Declaration, paragraph 16.

As noted above, neither Larsen nor Cummings teach or suggest treating or inhibiting thrombosis in a subject with hypertension and Blann, Araneo, DeFrees or the Merck Manual do not compensate for this deficiency, since none of these documents discuss administering a PSGL-1 protein for treating or inhibiting thrombosis in a subject having hypertension. Similarly, neither Maugeri nor Johnston compensate for these deficiencies because they also fail to discuss treating or preventing thrombosis in a subject having hypertension using a P-selectin ligand protein. To one of skill in the art, Maugeri and Johnston would not render the claimed invention obvious. *See* Hemmerich Declaration, paragraph 17.

Accordingly, Appellants respectfully request the withdrawal of the rejection of claim 27.

VIII. Conclusion

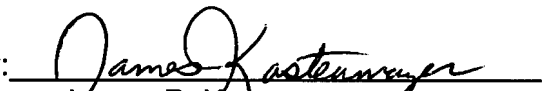
For the reasons given above, pending claims 1-20, 25-27, 31-40, and 45, and 50-57 are allowable and reversal of the Examiner's rejection is respectfully requested.

To the extent any extension of time under 37 C.F.R. § 1.136 is required to obtain entry of this Appeal Brief, such extension is hereby respectfully requested. If there are any fees due under 37 C.F.R. §§ 1.16 or 1.17 which are not enclosed herewith, including any fees required for an extension of time under 37 C.F.R. § 1.136, please charge such fees to our Deposit Account No. 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, L.L.P.

Dated: April 27, 2007

By: 
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IX. Claims Appendix to Appeal Brief Under Rule 41.37(c)(1)(viii)

1. (Previously presented) A method of treating or inhibiting thrombosis in a subject having hypertension comprising administering to the subject a composition comprising an effective amount of a PSGL-1 protein having a P-selectin ligand activity chosen from at least one of:

a) inhibiting P-selectin or E-selectin binding;

b) inhibiting adhesion of at least one of leukocytes to endothelial cells, leukocytes to platelets, leukocytes to blood vessels, and platelets to blood vessels;

c) inhibiting leukocyte recruitment to platelets and endothelial cells;

d) increasing leukocyte migration;

(e) increasing movement of at least one of leukocytes and platelets relative to blood vessels; and

f) increasing leukocyte rolling velocity.

2. (Previously presented) The method of claim 1, wherein the PSGL-1 protein is a soluble PSGL-1 protein or a fragment thereof having a P-selectin ligand activity.

3. (Original) The method of claim 2, wherein the soluble PSGL-1 protein is human PSGL-1.

4. (Original) The method of claim 2, wherein the soluble PSGL-1 protein is a recombinant protein.

5. (Original) The method of claim 2, wherein the soluble PSGL-1 protein comprises an Fc portion of an immunoglobulin.

6. (Original) The method of claim 5, wherein the immunoglobulin is human IgG1.

7. (Original) The method of claim 2, wherein the soluble PSGL-1 protein is a recombinant human PSGL-Ig fusion protein.

8. (Previously presented) The method of claim 2, wherein the soluble PSGL-1 protein comprises an extracellular domain of human PSGL-1 protein or a fragment thereof, capable of treating or inhibiting thrombosis.

9. (Original) The method of claim 8, wherein the fragment comprises the amino acid sequence set forth in SEQ ID NO:2 from amino acid 42 to amino acid 60.

10. (Original) The method of claim 8, wherein the fragment comprises the amino acid sequence set forth in SEQ ID NO:2 from amino acid 42 to amino acid 88.

11. (Original) The method of claim 8, wherein the fragment comprises the amino acid sequence set forth in SEQ ID NO:2 from amino acid 42 to amino acid 118.

12. (Original) The method of claim 8, wherein the fragment comprises the amino acid sequence set forth in SEQ ID NO:2 from amino acid 42 to amino acid 189.

13. (Original) The method of claim 8, wherein the fragment comprises the amino acid sequence set forth in SEQ ID NO:2 from amino acid 42 to amino acid 310.

14. (Original) The method of claim 2, wherein the soluble PSGL-1 protein comprises the amino acid sequence from amino acid 42 to amino acid 88 of SEQ ID NO:2 fused at its C-terminus to an Fc portion of an immunoglobulin.

15. (Original) The method of claim 8, wherein the soluble PSGL-1 protein further comprises an Fc portion of an immunoglobulin.

16. (Original) The method of claim 1, wherein the subject is human.

17. (Previously presented) The method of claim 1, wherein the PSGL-1 protein is administered to the subject prior to thrombus formation.

18. (Original) The method of claim 2, wherein the effective amount of soluble PSGL-1 protein or fragment thereof is between approximately 0.1 mg/kg and 10 mg/kg.

19. (Original) The method of claim 18, wherein the effective amount of soluble PSGL-1 protein is approximately 1 mg/kg.

20. (Previously presented) The method of claim 18, wherein the effective amount of soluble PSGL-1 protein is chosen from 0.1 mg/kg, 0.25 mg/kg, 0.5 mg/kg, 0.75 mg/kg, 1.0 mg/kg, 1.25 mg/kg, 1.5 mg/kg, 1.75 mg/kg, 2.0 mg/kg, 2.25 mg/kg, 2.5 mg/kg, 3.0 mg/kg, and 3.5 mg/kg.

21-24. (Canceled)

25. (Previously presented) A method for inhibiting thrombus formation induced by a thrombus-inducing agent in a subject comprising identifying a subject having hypertension and administering to the subject a composition comprising an effective amount of soluble PSGL-1 protein or a fragment thereof having a P-selectin ligand activity chosen from at least one of:

a) inhibiting P-selectin or E-selectin binding;

b) inhibiting adhesion of at least one of leukocytes to endothelial cells, leukocytes to platelets, leukocytes to blood vessels, and platelets to blood vessels;

c) inhibiting leukocyte recruitment to platelets and endothelial cells;

d) increasing leukocyte migration;

e) increasing movement of at least one of leukocytes and platelets relative to blood vessels; and

f) increasing leukocyte rolling velocity.

26. (Previously presented) The method of claim 25, wherein the soluble PSGL-1 protein or fragment thereof having a P-selectin ligand activity comprises a non-PSGL-1 amino acid sequence.

27. (Original) The method of claim 25, wherein the thrombus-inducing agent is LTC_4.

28. (Canceled)

29. (Withdrawn) The method of claim 1, wherein the subject has a condition chosen from prolonged sitting, bed rest and immobilization.

30. (Withdrawn) The method of claim 1, wherein the subject is at risk of thrombosis due to a vascular procedure chosen from angioplasty, surgical revascularization, balloon angioplasty, laser angioplasty, percutaneous transluminal coronary angioplasty, coronary artery bypass grafting, rotational atherectomy, stenting, and coronary artery stenting.

31. (Previously presented) A method of preventing or treating deep vein thrombosis, comprising identifying a subject having or at risk for deep vein thrombosis and administering to a subject a composition comprising an effective amount of a soluble PSGL-1 protein or a fragment thereof having a P-selectin ligand activity chosen from at least one of:

a) inhibiting P-selectin or E-selectin binding;

b) inhibiting adhesion of at least one of leukocytes to endothelial cells, leukocytes to platelets, leukocytes to blood vessels, and platelets to blood vessels;

c) inhibiting leukocyte recruitment to platelets and endothelial cells;

d) increasing leukocyte migration;

e) increasing movement of at least one of leukocytes and platelets relative to blood vessels; and

f) increasing leukocyte rolling velocity.

32. (Previously presented) The method of claim 31, wherein the soluble PSGL-1 protein or fragment thereof is a human PSGL-1.

33. (Previously presented) The method of claim 31, wherein the soluble PSGL-1 protein or fragment thereof comprises an extracellular domain of human PSGL-1 protein.

34. (Previously presented) The method of claim 31, wherein the soluble PSGL-1 protein or fragment thereof comprises amino acid 42 to amino acid 60 of SEQ ID NO:2.

35. (Previously presented) The method of claim 31, wherein the soluble PSGL-1 protein or fragment comprises the amino acid sequence set forth in SEQ ID NO:2 from amino acid 42 to amino acid 88.

36. (Previously presented) The method of claim 31, wherein the soluble PSGL-1 protein or fragment thereof comprises a non-PSGL-1 amino acid sequence.

37. (Previously presented) The method of claim 36, wherein the non-PSGL-1 amino acid sequence comprises an Fc portion of an immunoglobulin.

38. (Previously presented) The method of claim 37, wherein the soluble PSGL-1 protein or fragment comprises the amino acid sequence from amino acid 42 to amino acid 60 of SEQ ID NO:2.

39. (Previously presented) The method of claim 37, wherein the soluble PSGL-1 protein or fragment comprises the amino acid sequence from amino acid 42 to amino acid 88 of SEQ ID NO:2.

40. (Previously presented) The method of claim 31, wherein the subject is at risk for deep vein thrombosis due to hypertension.

41-42.(Canceled)

43. (Withdrawn) The method of claim 31, wherein the subject is at risk for deep vein thrombosis due to prolonged sitting, bed rest or immobilization.

44. (Withdrawn) The method of claim 31, wherein the subject is at risk for deep vein thrombosis due to a vascular procedure chosen from angioplasty, surgical revascularization, balloon angioplasty, laser angioplas ty, percutaneous transluminal coronary angioplasty, coronary artery bypass grafting, rotational atherectomy, stenting, and coronary artery stenting.

45. (Previously presented) A prophylactic method of treating or inhibiting thrombosis in a human subject comprising identifying a subject at risk of thrombosis due to hypertension and administering to the subject a composition comprising an effective amount of a PSGL-1 protein or a fragment thereof having a P-selectin ligand activity chosen from at least one of:

a) inhibiting P-selectin or E-selectin binding;

b) inhibiting adhesion of at least one of leukocytes to endothelial cells, leukocytes to platelets, leukocytes to blood vessels, and platelets to blood vessels;

c) inhibiting leukocyte recruitment to platelets and endothelial cells;

d) increasing leukocyte migration;

e) increasing movement of at least one of leukocytes and platelets relative to blood vessels; and

f) increasing leukocyte rolling velocity.

46. (Withdrawn) The method of claim 45, wherein the subject is at risk of thrombosis due to a disorder, condition or procedure chosen from:

(a) a cardiovascular disease or disorder;

(b) prolonged sitting, bed rest, or immobilization; and

(c) a surgical procedure.

47. (Withdrawn) The method of claim 46, wherein the cardiovascular disease or condition is chosen from hypertension, arterial inflammation, rapid ventricular pacing, aortic bending, vascular heart disease, atrial fibrillation, congestive heart failure, sinus node dysfunction, angina, heart failure, atrial flutter, cardiomyopathy, coronary artery disease, coronary artery spasm, and arrhythmia.

48. (Withdrawn) The method of claim 46, wherein the subject is at risk of thrombosis due to immobilization due to medical or surgical illness.

49. (Withdrawn) The method of claim 46, wherein the surgical procedure is chosen from a vascular procedure, angioplasty, surgical revascularization, balloon

angioplasty, laser angioplasty, percutaneous transluminal coronary angioplasty, coronary artery bypass grafting, rotational atherectomy, stenting, and coronary artery stenting.

50. (Previously presented) The method of claim 45, wherein the soluble PSGL-1 protein is a human PSGL-1.

51. (Previously presented) The method of claim 45, wherein the soluble PSGL-1 protein or fragment thereof comprises an extracellular domain of human PSGL-1 protein.

52. (Previously presented) The method of claim 45, wherein the soluble PSGL-1 protein or fragment thereof comprises amino acid 42 to amino acid 60 of SEQ ID NO:2.

53. (Previously presented) The method of claim 45, wherein the soluble PSGL-1 protein or fragment thereof comprises amino acid 42 to amino acid 88 of SEQ ID NO:2.

54. (Previously presented) The method of claim 45, wherein the soluble PSGL-1 protein or fragment thereof comprises a non-PSGL-1 amino acid sequence.

55. (Previously presented) The method of claim 54, wherein the non-PSGL-1 amino acid sequence comprises an Fc portion of an immunoglobulin.

56. (Previously presented) The method of claim 55, wherein the soluble PSGL-1 protein or fragment comprises the amino acid sequence from amino acid 42 to amino acid 60 of SEQ ID NO:2.

57. (Previously presented) A method for treating, inhibiting, or preventing thrombosis in a subject at risk of thrombosis comprising identifying a human subject at risk of

thrombosis due to hypertension and administering to the subject a composition comprising an effective amount of a soluble PSGL-1 protein or fragment thereof having a P-selectin activity chosen from at least one of:

a) inhibiting P-selectin or E-selectin binding;

b) inhibiting adhesion of at least one of leukocytes to endothelial cells, leukocytes to platelets, leukocytes to blood vessels, and platelets to blood vessels;

c) inhibiting leukocyte recruitment to platelets and endothelial cells;

d) increasing leukocyte migration;

e) increasing movement of at least one of leukocytes and platelets relative to blood vessels; and

f) increasing leukocyte rolling velocity.

X. Evidence Appendix to Appeal Brief Under Rule 41.37(c)(1)(ix)

Appellants rely on the Declaration of Dr. Stefan Hemmerich, submitted on September 13, 2006, in support of these arguments. The Examiner entered this Declaration into the record on September 28, 2006, and a copy has been included with this filing. Additionally, Appellants rely on the following publications discussed in and attached to this Declaration:

- Cummings *et al.* U.S. Patent No. 5,464,778;
- THE MERCK MANUAL OF DIAGNOSIS AND THERAPY 1655 (17th ed. 1999);
- Larsen *et al.*, U.S. Patent No. 5,840,679;
- Lip *et al.*, "Hypertension and the prothrombotic state," J. Hum. Hyper. 14: 687-90 (2000);
- Blann *et al.*, "Evidence of platelet activation in hypertension," J. Hum. Hyper. 11:607-609 (1997);
- Araneo *et al.*, U.S. Patent No. 6,150,348;
- DeFrees *et al.*, U.S. Patent No. 5,604,207;
- Maugeri *et al.*, "Polymorphonuclear Leukocyte-Platelet Interaction: Role of P-selectin in Thromboxane B_2 and Leukotriene C_4 Cooperative Synthesis," *Thromb. Haem.* 72:450-456 (1994); and
- Johnston *et al.*, "Differential Roles of Selectins and the α4-Integrin in Acute, Subacute, and Chronic Leukocyte Recruitment In Vivo," *J. Immunol.* 159:4514-4523 (1997).

XI. Related Proceedings Appendix to Appeal Brief Under Rule 41.37(c)(1)(x)

Appellants are not aware of or relying on any decisions in related proceedings.

FOREIGN LANGUAGE EDITIONS
of The Merck Manual

Arabic—Larike Publications Services, Cyprus
Chinese—People's Medical Publishing House, Beijing
Czech—Egem, Prague
French—Editions d'Apres, Paris
German—Urban & Schwarzenberg, GmbH, Munich
Greek—Medical & Scientific Publishing, Athens
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Italian—Stampa Medica, s.p.a., Rome
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Russian—MIR Publishers, Moscow
Spanish—Harcourt Brace, Barcelona
Turkish—Yüce, Istanbul

OTHER MERCK BOOKS

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First Edition, 1955

THE MERCK MANUAL OF GERIATRICS
First Edition, 1990

THE MERCK MANUAL OF MEDICAL INFORMATION—HOME EDITION
First Edition, 1997

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FOREWORD

With this edition, *The Merck Manual* celebrates its 100th birthday. When the editors of the 1st Edition produced their 192-page compendium, they could not have realized the extent to which medical knowledge would explode over the next century. *The Merck Manual* now fills 2,655 pages and covers countless diseases that were not known 100 years ago. A brief review of medical practice as reflected in *The Merck Manual* during the past century follows on page vii.

Although the knowledge of medicine has grown, the goal of *The Merck Manual* has not changed—To provide useful clinical information to practicing physicians, medical students, interns, residents, nurses, pharmacists, and other health care professionals in a concise, complete, and accurate manner. *The Merck Manual* continues to cover all the subjects expected in a textbook of internal medicine as well as detailed information on pediatrics, psychiatry, obstetrics, gynecology, dermatology, pharmacology, ophthalmology, otolaryngology, and a number of special subjects. *The Merck Manual* quickly provides information that helps practitioners achieve optimal care. The more specialized the practice of medicine becomes, the more important such information becomes. Specialists as well as generalists must at some time quickly access information about other specialties.

The 17th edition of *The Merck Manual* is the culmination of an arduous but rewarding 7-year enterprise. Every topic has been updated, and many have been completely rewritten. Topics new to this edition include hand disorders, prion diseases, death and dying, probabilities in clinical medicine, multiple chemical sensitivity, chronic fatigue syndrome, rehabilitation, smoking cessation, and drug therapy in the elderly, among others. The members of the Editorial Board, special consultants, and contributing authors are listed on the following pages with their affiliations. They deserve a degree of gratitude that cannot be adequately expressed here, but we know they will feel sufficiently rewarded if their efforts serve your needs.

Because of the extensive subject matter covered and a successful tradition developed through trials of successes and failures, *The Merck Manual* has some unique characteristics. We urge readers to spend a few minutes reviewing the Guide for Readers (p. xii), the Table of Contents at the beginning of each section (indicated by a thumb tab), and the Index (p. 2657). Subject headings within each section, internal headings within a subject discussion, and boldfaced terms in the text form an outline intended to help with use of the text.

We hope this edition of *The Merck Manual* will serve as an aid to you, our readers, compatible with your needs and worthy of frequent use. Suggestions for improvements will be warmly welcomed and carefully considered.

MARK H. BEERS, M.D., and ROBERT BERKOW, M.D., *Editors*

geal drying within the first 2 wk. Long-term compliance is the major problem with nasal CPAP. About 70% of patients use CPAP > 2 yr. Patients with claustrophobia are disinclined to use CPAP but may be able to tolerate it with practice.

Removable dental appliances worn when sleeping may benefit persons with obstructive sleep apnea. Some are designed to keep the soft palate elevated; others keep the tongue protruded. Still others separate the jaws and position the mandible anteriorly so the tongue cannot move backward to obstruct the pharynx. The appliances are generally well tolerated and may obviate the need for surgery. Effectiveness, discomfort, possible other complications, and long-term compliance should be evaluated frequently.

Surgery is rarely needed. Few patients (eg, those who have severe heart failure or severe pulmonary disease, who cannot tolerate CPAP, and for whom other measures failed) require tracheostomy. Uvulopalatopharyngoplasty to enlarge the pharyngeal airspace has been attempted but is successful in only about half the cases. Relieving the obstruction usually reverses associated pulmonary and systemic hypertension, cardiac arrhythmias, and cognitive difficulties.

For **snoring**, avoiding alcoholic beverages, tranquilizers, sleeping pills, and antihistamines before retiring; sleeping prone or on one's side; or raising the head of the bed may help. Special antisnoring pillows are no more effective than regular pillows or raising the head of the bed. The various devices promoted to reduce snoring usually work well only in mild cases and do not relieve sleep apnea. Nasal infections and allergies should be treated. For heavy snoring, surgically correcting obstructive conditions in the nose, pharynx, or uvula (eg, by laser-assisted uvulopalatoplasty) may be the only solution if treatment is needed.

PARASOMNIAS

Somnambulism is sitting, walking, or performing other complex behavior during sleep, usually with the eyes open but without evidence of recognition. The condition is most common during late childhood and adolescence. Patients may mumble repetitiously, and some injure themselves on obstacles or stairs. There is no accompanying dream. Usually, the patient does not remember the episode. Treatment is directed at protecting the person from injury and dealing with any underlying disorder. Benzodiazepines, particularly diazepam and alprazolam, may help. Other drugs, such as selective serotonin reuptake inhibitors, can be considered for severe cases refractory to benzodiazepines.

Night terrors (fearful, screaming, flailing episodes) are more common in children than in adults and are often accompanied by sleepwalking. They occur during NREM stages 3 and 4 sleep. In adults, night terrors are often associated with psychologic difficulties or alcoholism. Intermediate- or long-acting benzodiazepines, such as diazepam 2 to 5 mg, at bedtime sometimes prevent episodes.

Nightmares (frightening dreams) affect children more frequently than adults. They occur during REM sleep, more commonly with fever or excess fatigue or after alcohol ingestion. Treatment is directed at the underlying conflicts or disorder.

Restless legs syndrome is a relatively common disorder that often occurs just before falling asleep, particularly among persons > 50 yr. The cause is unknown, but 1/3 of persons with the syndrome have a family history. Uncomfortable sensations that are difficult to describe are felt in the legs and are relieved temporarily by movement. Patient distress and sleep loss may become severe. Treatment can be difficult and often requires trying different drugs and dosage regimens. The drugs of choice are the dopamine agonists pergolide and carbidopa-levodopa. Other choices are oxycodone, clonazepam, and gabapentin. Benzodiazepines taken at bedtime prevent awakenings but not nocturnal movements.

Nocturnal leg cramps commonly occur in otherwise healthy middle-aged and elderly patients during sleep. They affect the calf muscles, causing forceful plantar flexion of the foot or toes. Diagnosis is based on the history and lack of physical signs on examination. Stretching the affected muscles for several minutes before sleep often helps prevent cramps. Stretching immediately after cramping usually relieves symptoms and is preferable to empiric drug treatment. Quinine sulfate 200 to 300 mg at bedtime is used, but recent studies claim that it is not effective for night cramps. In large doses, it causes a bitter taste, tinnitus, flushing, pruritus, and GI disturbances, and it interacts with many other drugs. Calcium supplements (eg, calcium gluconate 1 to 2 g bid) are well tolerated, but their effectiveness is doubtful. Drugs used with quinine and calcium include diphenhydramine 50 to 100 mg at bedtime, magnesium oxide 100 to 200 mg bid, and low doses of benzodiazepines. However, with all these drugs, toxic effects can outweigh any benefit. Mexiletine 150 mg tid is sometimes effective when increased irritability of the lower motor neuron is suspected. Avoiding caffeine and other sympathetic stimulants may help.

174 / CEREBROVASCULAR DISEASE

(Stroke; Cerebrovascular Accident)

In Western countries, stroke is the third most common cause of death and the second most common cause of neurologic disability after Alzheimer's disease. Its incidence has decreased in recent decades, but the decrease appears now to have leveled off, and cerebrovascular disease remains the leading cause of institutional placement for loss of independence among adults.

Most vascular injury to the brain is secondary to atherosclerosis or hypertension. The major types of cerebrovascular disease are cerebral insufficiency due to transient disturbances of blood flow or, rarely, hypertensive encephalopathy; infarction due to embolism or thrombosis of intracranial or extracranial arteries; hemorrhage, including hypertensive parenchymal hemorrhage and subarachnoid hemorrhage due to congenital aneurysm; and arteriovenous malformation, which can cause symptoms of a mass lesion, infarction, or hemorrhage.

Symptoms and signs in cerebrovascular disease reflect the damaged area of brain and not necessarily the affected artery. For example, occlusion of either the middle cerebral or internal carotid artery can produce a similar clinical neurologic abnormality. Nevertheless, cerebrovascular injuries generally conform to fairly specific patterns of arterial supply; knowledge of these patterns helps distinguish stroke from other brain lesions that occasionally produce acute symptoms (see TABLE 174–1).

TABLE 174–1. DIFFERENTIAL DIAGNOSIS FOR STROKE

Brain tumor
Cerebral hypoxia
Cranial or peripheral nerve palsy
Functional disorder
Hypoglycemia
Migraine
Multiple sclerosis
Peripheral vascular disease
Seizure
Subdural hematoma
Syncope or near syncope

An accurate history, including onset and duration of symptoms and identification of stroke risk factors, is key to diagnosing cerebrovascular lesions (see TABLE 174–2).

TABLE 174–2. STROKE RISK FACTORS

Untreatable	Treatable	
> 60 yr	Cardiac arrhythmia	Hypertension
Family history of stroke	Cardiomyopathy	Hyperviscosity state
Male sex	Diabetes mellitus	Illicit drug use
Prior transient ischemic attack or stroke	Excessive ethanol use	Oral contraceptive use
	History of migraine	Tobacco use
	Hypercoagulable state	Valvular heart disease
	Hyperlipidemia	Vasculitis

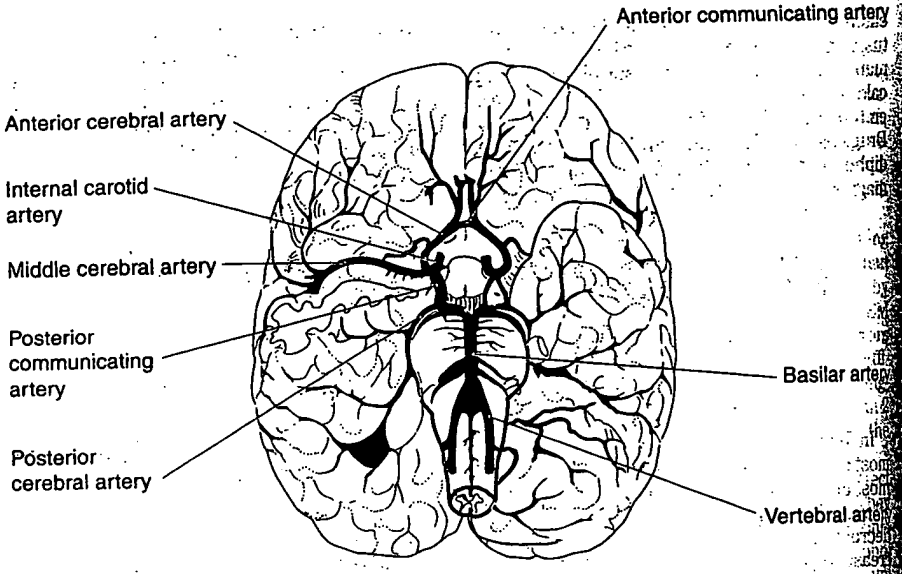

FIG. 174–1. Arteries of the brain.

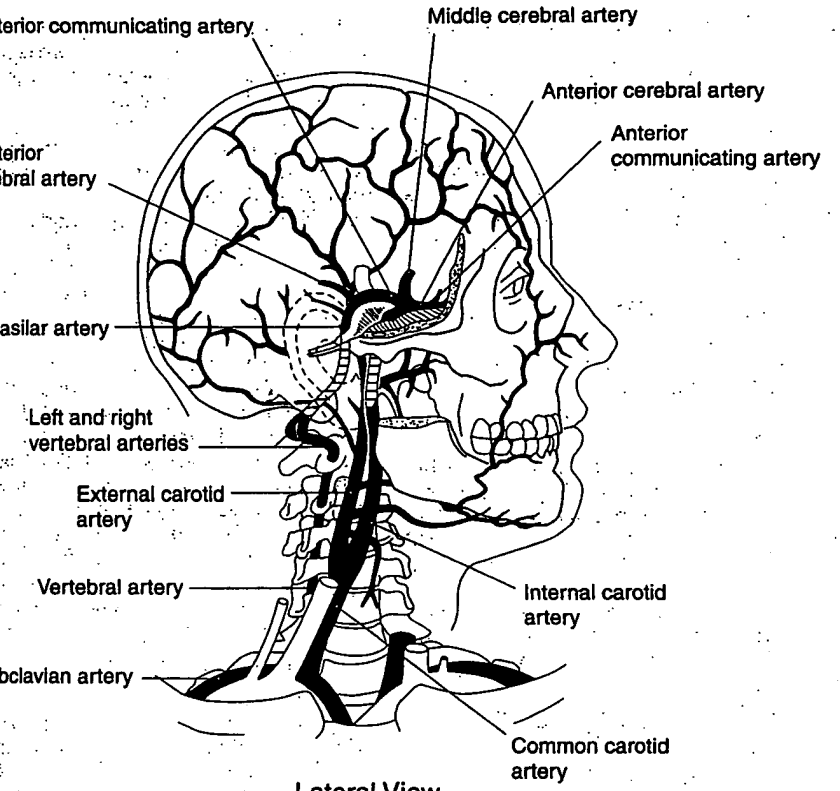

FIG. 174–1. *Continued*

Hemorrhagic stroke has a more catastrophically acute onset than ischemic stroke, although both tend to develop abruptly. A brain CT or MRI scan can distinguish between ischemic and hemorrhagic strokes, thus assisting in urgent treatment decisions.

Various standardized tests are used to assess the severity of stroke. For example, the National Institutes of Health Stroke Scale assesses consciousness, vision, extraocular movements, facial palsy, limb strength, ataxia, sensation, speech, and language using 15 items scored from 0 to 2 or 3. Higher scores reflect increased severity of the deficit; the highest possible total score is 42.

ISCHEMIC SYNDROMES

Cerebrovascular disorders caused by insufficient cerebral circulation.

Syndromes include transient ischemic attacks (TIAs) and ischemic stroke.

Normally, adequate cerebral blood supply is ensured by an efficient collateral system: from one vertebral artery to another, between the carotid and vertebral arteries via the anastomoses at the circle of Willis, and through collateral circulation at the level of the hemispheres. Congenital anomalies and atherosclerosis can interrupt intracranial or extracranial arterial blood flow and impair collateral flow, causing brain ischemia and consequent neurologic symptoms. If blood supply is promptly restored, brain tissues recover and symptoms disappear, but if ischemia lasts longer than 1 h, infarction and permanent neurologic damage result.

Thrombi or emboli due to atherosclerosis or other disorders (eg, arteritis, rheumatic heart disease) commonly cause ischemia by arterial obstruction. Atheromas, which underlie most thrombi, may affect any major cerebral artery (see FIG. 174–1). Large atheromas usually affect the common carotid and vertebral arteries at their origins, but the cervical bifurcation of the common carotid artery is the most common site giving rise to emboli that cause strokes. Intracranial thrombosis may occur in one of the large arteries at the base of the brain, in a deep perforating artery, or in a small cortical branch, but the main trunk of the middle cerebral artery and its branches are the most common sites. The intracranial carotid siphon and the basilar artery just proximal to the origin of the posterior cerebral artery are often affected. Whether ischemia and/or infarction occurs depends on the efficiency of collateral circulation; eg, concomitant stenosis of both vertebral arteries can compromise collateral circulation and intensify the effects of carotid lesions.

Less commonly, vascular inflammation causes thrombotic occlusion, secondary to such disorders as acute or chronic meningitis, collagen vascular disease, or syphilis.

Cerebral emboli may lodge temporarily or permanently anywhere in the cerebral arterial tree. They usually come from atheromas in extracranial vessels or from thrombi in a damaged heart, especially from vegetations on the heart valves in bacterial or marantic endocarditis, from mural thrombi in atrial fibrillation or after MI, or from clots after open-heart surgery. Rarely, emboli are fat (from fractures of long bones), air (caisson disease), or venous clots that pass from the right to the left side of the heart through a patent foramen ovale (paradoxical embolus). Cerebral emboli may result from atherosclerosis of the aortic arch. Emboli may also occur spontaneously or be dislodged by invasive cardiovascular procedures (eg, after aortic catheterization).

Physiologic circulatory insufficiency is a relatively uncommon cause of ischemia and infarction. Diminished perfusion may occur alone or be superimposed on an existing partial occlusion. Many processes can reduce perfusion. Profound anemia and carbon monoxide poisoning (by reducing the O_2-carrying capacity of the blood) and severe polycythemia (by increasing the viscosity of blood) can contribute to cerebrovascular problems. Usually, a fall in arterial pressure must be pronounced and sustained to severely compromise regional blood flow, but if arterial disease or hypoxemia is present, a

lesser fall in BP can cause ischemia and infarction.

Ischemia may result from using sympathomimetic drugs (eg, cocaine, amphetamine), presumably through a vasculitic mechanism. Ischemic stroke was associated with use of older oral contraceptives; the association with current, lower-dose contraceptives is weaker. Very rarely, bony vertebral projections (osteophytes) cause arterial compression.

TRANSIENT ISCHEMIC ATTACKS

Focal neurologic abnormalities of sudden onset and brief duration that reflect dysfunction in the distribution of the internal carotid–middle cerebral or the vertebrobasilar arterial system.

Most TIAs are due to cerebral emboli from ulcerated atherosclerotic plaques in the carotid or vertebral arteries in the neck or, less often, from mural thrombi in a diseased heart. Some TIAs are due to a brief reduction in blood flow through stenosed arteries.

Hypertension, atherosclerosis, heart disease, atrial fibrillation, diabetes mellitus, and polycythemia predispose to TIAs. TIAs are most common in the middle-aged and elderly but occasionally occur in children with severe cardiovascular disease that produces emboli or a very high Hct.

In the **subclavian steal syndrome**, a rare condition, a subclavian artery stenosed proximal to the origin of the vertebral artery "steals" reverse-flow blood from the vertebral artery to supply the arm during exertion. Angiographic evidence of reversed flow between the vertebral artery and the stenosed subclavian artery is never diagnostic in the absence of clinical signs indicating vertebral-basilar ischemia caused by exertion of the affected arm.

Symptoms and Signs

TIAs begin suddenly, last 2 to 30 min or more (seldom > 1 or 2 h), then abate without persistent neurologic abnormalities; consciousness remains intact throughout the episode. When TIAs last for hours, patients may have infarcts, seen on subsequent brain CT or MRI scans, even without persistent neurologic abnormalities.

Symptoms are identical to those of stroke but are transient. With carotid artery involvement, symptoms are generally unilateral. Ipsilateral blindness or contralateral hemiparesis, often with paresthesias, is classic, but less complete symptoms are more common. Aphasia indicates involvement of the dominant hemisphere. When the vertebrobasilar system (which supplies the brain stem, cerebellum, and portions of the temporal and occipital lobes) is affected, symptoms reflect brain stem dysfunction. Confusion, vertigo, binocular blindness, diplopia, and unilateral or, more often, bilateral weakness or paresthesias of the extremities may be present. Slurred speech (dysarthria) may occur with carotid or vertebrobasilar involvement.

Drop attacks, in which a conscious patient's legs buckle, usually precipitating a fall, are often attributed to vertebrobasilar ischemia, but the actual cause of this common condition is uncertain.

Patients may have several TIAs daily or only two or three over several years. Symptoms are usually similar in successive carotid attacks but vary somewhat in successive vertebrobasilar attacks. Patients with TIAs are at a markedly increased risk of stroke and should be evaluated for possible causes on an urgent basis.

Diagnosis and Treatment

Differentiation from convulsive seizures, neoplasms, migraine, Meniere's disease, other forms of vertigo, and hyperinsulinism in diabetics is sometimes necessary. Noninvasive ultrasonography, magnetic resonance angiography, or invasive arteriography can confirm the presence of stenosis and identify the affected artery; such confirmation is needed when surgery of carotid arteries is contemplated. Concomitant subclavian artery occlusion is indicated by brachial BP that is significantly lower in the affected arm than in the opposite arm. If a cardioembolic source is suspected, echocardiography should be performed.

Underlying risk factors (see TABLE 174-2) should be identified and treated if possible.

If patients with carotid TIAs have a documented obstruction of > 70% or an ulcerated plaque in the ipsilateral carotid artery, endarterectomy significantly reduces the chance of a stroke compared with medical therapy alone. For an obstruction of < 30%, medical therapy is preferred. For an obstruction between 30 and 70%, the best therapy has not been determined. Several randomized, multicenter trials indicate that endarterectomy reduces the risk of TIA and stroke in asymptomatic patients with carotid artery stenosis of > 60%. Nevertheless, endarterectomy for such patients is controversial because the risk/benefit ratio is narrow and depends on patient selection and surgical morbidity and mortality rates of < 3%.

Antiplatelet drugs or anticoagulants are used when the obstruction is intracranial or vertebrobasilar or when both vertebral and carotid arteries are affected, provided the patient is not hypertensive. Heparin is used initially for recent daily attacks; a warfarin derivative can be used for less frequent attacks. The duration of anticoagulant therapy is empiric; often, anticoagulants are continued for 2 to 3 mo before a trial without therapy. For patients with occasional TIAs secondary to atherothrombosis, most authorities try antiplatelet drugs before starting anticoagulants. Unless specifically contraindicated, antiplatelet drugs should be continued indefinitely. Aspirin 650 to 1300 mg/day or ticlopidine 250 mg bid is the drug of choice; the optimal dosage for aspirin is unknown. The usefulness of sulfinpyrazone, dipyridamole, and clofibrate has not been established.

Surgical anastomosis or bypass between the external carotid and middle cerebral artery is generally not beneficial, but a bypass may benefit selected patients who require immediate carotid occlusion or who have occlusion with inadequate collateral flow and symptoms despite anticoagulation.

ISCHEMIC STROKE

Stroke in evolution (evolving stroke): *An enlarging brain infarct manifested by neurologic deficits that worsen over 24 to 48 h.* ***Completed stroke:*** *Brain infarct manifested by neurologic deficits that signify stable injury.*

Typically, strokes are caused by arteriosclerotic or hypertensive stenosis, thrombosis, or embolism.

Symptoms and Signs

Onset is abrupt. In evolving stroke, unilateral neurologic dysfunction (often beginning in one arm, then spreading progressively and ipsilaterally) extends painlessly over several hours to a day or two, without producing headache or fever. Progression is usually stepwise, interrupted by periods of stability, but may be continuous.

Acute completed stroke is more common. Symptoms develop rapidly, typically becoming maximal within a few minutes. An evolving stroke may become a completed stroke.

During the first 48 to 72 h of an evolving stroke or of a large completed stroke, deficits may worsen and consciousness become clouded because of cerebral edema or, less often, extension of the infarct. Severe cerebral edema can cause a potentially fatal shift in intracranial structures (transtentorial herniation; see INTRACRANIAL NEOPLASMS in Ch. 177). However, unless the infarct is large or extensive, function commonly improves early, with further improvement occurring gradually over days to months.

The **middle cerebral artery** or one of its deep penetrating branches is most commonly occluded. Occlusion of the proximal part of the artery, which supplies large portions of the frontal, parietal, and temporal lobe surfaces, results in contralateral hemiplegia (usually severe), hemianesthesia, and homonymous hemianopia. Aphasia occurs when the dominant hemisphere is affected; apraxia and/or sensory neglect occurs when the nondominant hemisphere is affected. Contralateral hemiplegia of the face, arm, and leg, sometimes with hemianesthesia, also results from occlusion of one of the deep branches, which supply the basal ganglia, internal and external capsules, and thalamus. Motor or sensory impairment may be less severe when terminal branches are occluded.

Internal carotid artery occlusion leads to infarction in the central-lateral portion of the cerebral hemisphere, with symptoms identical to those of middle cerebral artery occlusion except for occasional ocular symptoms ipsilateral to the diseased internal carotid artery.

Anterior cerebral artery occlusion is uncommon. It affects the medial portions of the frontal and parietal lobes, corpus callosum, and sometimes the caudate nucleus and internal capsule. Contralateral hemiplegia (especially of the leg), a grasp reflex, and urinary incontinence may occur. Bilateral occlusion may cause spastic paraparesis and emotional disturbances with apathy, confusion, and occasional mutism.

Posterior cerebral artery occlusion can affect areas in the temporal and occipital lobes, internal capsule, hippocampus, thalamus, mammillary and geniculate bodies,

choroid plexus, and upper brain stem. Contralateral homonymous hemianopia, hemisensory loss, spontaneous thalamic pain, and sudden hemiballism may occur; alexia may follow an infarct in the dominant hemisphere.

Branch occlusions of the **vertebrobasilar system** cause combinations of cerebellar, corticospinal, sensory, and cranial nerve signs. With unilateral disease, cranial nerve abnormalities are often contralateral to the side of body weakness or sensory changes. Complete occlusion of the basilar artery usually causes ophthalmoplegia, pupillary abnormalities, bilateral corticospinal signs (tetraparesis or tetraplegia), and changes in consciousness. Pseudobulbar manifestations (dysarthria, dysphagia, emotional instability) occur often. Death often results.

Diagnosis

Ischemic stroke usually can be diagnosed clinically, especially in a person over age 50 with hypertension, diabetes mellitus, or signs of atherosclerosis or in a person with a condition that produces emboli. Carotid bruits and thrills in the neck may indicate stenosis and plaque formation; neurologic symptoms and signs can suggest the artery affected, although the correlation is inexact.

Determining the immediate cause of a stroke may be difficult. Onset during sleep or on arising suggests infarction; onset during exertion, hemorrhage. Headache, coma or stupor, marked hypertension, and convulsive seizures are more likely with hemorrhage. Concomitant signs of MI, atrial fibrillation, or vegetative heart disease suggest embolism. Neck pain with a new neurologic deficit suggests a dissection; dissections can occur without pain. A large embolus tends to cause an acute completed stroke, with sudden onset and focal disorders that are maximal within minutes; headache may precede the stroke. Thrombosis, a less common cause, is suggested by a slower onset or gradually progressing symptoms (as in evolving stroke), but the distinction is not reliable.

Laboratory studies should be performed to identify hypertension and to rule out anemia, polycythemia, hypercoagulable states, and infections. Plasma lipids should be determined, and an ESR may help exclude vasculitis. A Venereal Disease Research Laboratory (VDRL) test is desirable for persons at increased risk of syphilis. A chest x-ray should be taken to search for a primary lung tumor and cardiovascular disorders; an ECG should be performed.

In all forms of ischemic stroke, CSF is usually normal, but WBCs may transiently increase to 500/μL, glucose may decrease slightly, and protein may increase to 80 mg/dL. CSF is usually clear after an infarct but it is bloody and under increased pressure after intracranial hemorrhage. CSF may contain RBCs after infarction but far fewer than after hemorrhage.

Usually, a CT or MRI scan helps differentiate an ischemic stroke from intracerebral hemorrhage, hematoma, or a rapidly growing or suddenly symptomatic tumor. An MRI scan usually detects areas of evolving infarction within hours; a CT scan is sometimes negative for up to several days after acute infarction. Arteriography is performed only when the diagnosis is in doubt or when remedial vascular obstruction (eg, by surgery) is suspected. However, noninvasive studies, such as carotid duplex, ultrasonography, or magnetic resonance angiography may be useful.

Prognosis

During the first days of an ischemic stroke, neither progression nor outcome can be predicted. About 20% of patients die in the hospital; the mortality rate increases with age.

The extent of neurologic recovery depends on the patient's age and general state of health and on the site and size of the infarct. Impaired consciousness, mental deterioration, aphasia, or severe brain stem signs suggest a poor prognosis. Complete recovery is uncommon, but the sooner improvement begins, the better the prognosis. About 50% of patients with moderate or severe hemiplegia and most with milder deficits recover functionally by the time of discharge and can eventually care for their basic needs, have a clear sensorium, and can walk adequately, although use of an affected limb may be limited. Any deficit remaining after 6 mo is likely to be permanent, although some patients continue to improve slowly. Cerebral infarction recurs relatively often, and each recurrence is likely to add to the neurologic disability.

Treatment

Immediate care of a comatose patient includes airway maintenance, adequate O_2 and fluids to maintain nutritional and fluid intake, attention to bladder and bowel function, and measures to prevent decubitus ulcers. *Corticosteroids are not indicated in the treatment of ischemic stroke.*

Heart failure, arrhythmias, severe hypertension (ie, systolic BP > 220 or diastolic BP > 120), intercurrent respiratory infection, and body temperature > 100° F (37.8° C) must be treated. IV spasmolytic drugs (eg, sodium nitroprusside) are preferable for malignant hypertension. *Barbiturates and other sedatives are contraindicated* because they increase the risk of respiratory depression and subsequent pneumonitis. Passive exercises, particularly of paralyzed limbs, and breathing exercises, if possible, should be started early.

Recombinant tissue plasminogen activator (tPA), given within 3 h of symptom onset, can improve neurologic outcome of selected acute stroke patients (see TABLE 174–3 for exclusion criteria). The dose of recombinant tPA is 0.9 mg/kg IV (maximum dose 90 mg); 10% is given by rapid IV injection, and the remainder by constant infusion over 60 min. Symptomatic and fatal hemorrhage is more common in patients receiving tPA than in those receiving placebo, but the overall mortality rates of the two groups are not different. Only physicians experienced in stroke management should use tPA for patients with acute stroke. Vital signs must be closely monitored for 24 h after treatment, and any bleeding complications aggressively managed. Anticoagulants and antiplatelet drugs should not be used within 24 h of treatment with tPA.

Anticoagulation with heparin may stabilize symptoms in patients with evolving stroke who are not candidates for tPA. However, whether anticoagulants should be given before stroke etiology has been determined is under study.

Guidelines for **anticoagulation** to prevent subsequent strokes, especially those secondary to cardioembolism or a hypercoagulable state, are well defined. Patients with small nonhemorrhagic infarcts of cardioembolic origin should be treated initially with heparin and then switched to warfarin, which should be continued for at least 6 mo or if rhythm abnormality or valvular disease persists, probably indefinitely. Concomitant heparin infusion should be used to increase partial thromboplastin time to 1.5 to 2.0 times control values until the prothrombin time reaches an INR of 2.0 to 3.0. Anticoagulation should be delayed for 5 to 7 days in patients with large nonhemorrhagic infarcts of cardioembolic origin and for 2 to 4 wk in patients with hemorrhagic infarcts of cardioembolic origin. Heparin (20,000 U in 500 mL 5% dextrose solution) should be

TABLE 174–3. EXCLUSION CRITERIA FOR USE OF TISSUE PLASMINOGEN ACTIVATOR IN STROKE*

Intracranial hemorrhage on CT scan
Patients with large, acute brain infarcts on CT or MRI scan
Minor stroke symptoms (eg, < 4 on the NIH Stroke Scale), rapidly improving symptoms, or severe symptoms (eg, > 22 on the NIH Stroke Scale)
Presentation suggesting subarachnoid hemorrhage even if CT scan is negative
History of intracranial hemorrhage, AVM, aneurysm, or brain tumor
History of stroke or head trauma within the past 3 mo
Systolic BP > 185 mm Hg or diastolic BP > 110 mm Hg (aggressive treatment to reduce BP to specified limits is required)
Arterial puncture at noncompressible site or lumbar puncture in the past 7 days
Major surgery or serious trauma in the past 14 days
GI or urinary tract hemorrhage in the past 21 days
Platelet count < 100,000
PTT elevated above control due to treatment with heparin within 48 h
Current use of oral anticoagulants, PT > 15, or INR > 1.7
Seizure at the onset of this stroke
Blood glucose < 50 or > 400 mg/dL (< 2.78 or > 22.2 mmol/L)
Recent MI, bacterial endocarditis, or pericarditis
Known or suspected pregnancy

*Initiation of treatment is required within 3 h of symptom onset.

NIH = National Institutes of Health; AVM = arteriovenous malformation; PTT = partial thromboplastin time; PT = prothrombin time; INR = international normalized ratio.

given IV via constant infusion pump; rapid IV injection to initiate or maintain heparin therapy is not recommended for stroke. Patients with a hypercoagulable condition should be given heparin and warfarin promptly. Heparin should be given until warfarin has increased the INR to 3.0 in patients with an elevated anticardiolipin antibody titer or a positive circulating lupus anticoagulant. Whether an antiplatelet drug is the best prophylaxis for atherothrombotic stroke is under study.

Vascular surgery is not indicated as an emergency measure and should be used after a completed hemiplegic stroke only if viable hemispheric tissue at risk for further injury and functional loss remains. The indications for prophylactic thromboendarterectomy are the same as those for endarterectomy in TIAs (see above).

Rehabilitation

Early, repeated appraisals of the patient's status by the physician, physiotherapist, and nursing staff guide rehabilitation (see also Ch. 291). Elaborate programs are unnecessary, and the value of speech therapy is unproved, especially early in recovery. Younger age, limited sensory and motor deficits, intact mental function, and a helpful home environment favorably influence rehabilitation. Early treatment, continuing encouragement, and training for real-life needs are important. The patient, relatives, and friends must understand what the disabilities are and that improvement is likely, but only with time, patience, and perseverance. Mood changes may be due to the infarct and to the patient's frustration at his condition and should be expected; caregivers should respond with reassurance and understanding. Sedatives or antidepressants may help after the patient's condition has stabilized. Occupational and physical therapy should emphasize using affected limbs and achieving proficiency in eating, dressing, toilet functions, and other basic needs. Appliances (eg, hearing aids, walking frames) are often needed; in the living quarters, hand bars (eg, at tub and toilet) and ramps can help.

Some patients are so severely affected that rehabilitation is unlikely to help; long-term care may be more appropriate for them. The dying should receive appropriate care to eliminate suffering (see Ch. 294). Enteral nutrition is discussed in Ch. 1.

HEMORRHAGIC SYNDROMES

Cerebrovascular disorders caused by bleeding into brain tissue; the epidural, subdural, or subarachnoid space; or a combination of these sites.

INTRACEREBRAL HEMORRHAGE

Intracerebral hemorrhage usually results from rupture of an arteriosclerotic vessel that has been long exposed to arterial hypertension or made ischemic by local thrombosis. Less often, the cause is a congenital aneurysm or other vascular malformation. Amyloid angiopathy may cause polar hemorrhages. Occasional causes include mycotic aneurysms, brain infarct, blood dyscrasias, collagen vascular diseases, and abuse of cocaine or other illicit drugs. Hypertensive intracerebral hemorrhage is usually large, single, and catastrophic.

Intracerebral hemorrhages can occur almost anywhere in the brain. Most clinically destructive are those located near the basal ganglia, internal capsule, thalamus, cerebellum, or brain stem.

A hematoma dissects, compresses, and displaces adjacent brain tissue and, if large, increases intracranial pressure. Pressure from supratentorial hematomas and the accompanying edema may cause transtentorial herniation, compressing the brain stem and often causing secondary hemorrhages in the midbrain and pons. If the hemorrhage ruptures into the ventricular system, blood may reach the subarachnoid space. Cerebellar hematomas can expand to block the ventricular system, causing acute hydrocephalus, or dissect into the brain stem. Either course can produce stupor or coma.

Symptoms and Signs

Symptoms typically begin abruptly with headache, followed by steadily increasing neurologic deficits. Large hemorrhages when located in the hemispheres, produce hemiparesis; when located in the posterior fossa, they produce symptoms of cerebellar or brain stem dysfunction (conjugate eye deviation or ophthalmoplegia, stertorous breathing, pinpoint pupils, and coma). Loss of consciousness is common, occurring within a few minutes after onset or developing gradually. Nausea, vomiting, delirium, and focal or generalized seizures are also common. Large hemorrhages are fatal within a few days in > 50% of patients. In survivors, consciousness returns and neurologic deficits gradually diminish as the extravasated blood is resorbed. Some degree of impairment usually remains, including some dysphasia if the dominant hemisphere was affected, but many patients make a reasonable functional recovery, especially from hemorrhages in silent areas. Small hemorrhages cause focal deficits like those in ischemic stroke.

Diagnosis and Treatment

Clinically, distinguishing small intracerebral hemorrhages from ischemic stroke is often difficult (see also ISCHEMIC STROKE, above). CT is the procedure of choice because hemorrhage is so easily seen as hyperintensity. MRI also helps distinguish hemorrhagic from ischemic strokes, but its logistics and the need for experienced interpreters of the image make MRI less practical than CT. Lumbar puncture must be performed cautiously, if at all, when patients are unconscious or symptoms are worsening, because the consequent change in CSF pressure may precipitate transtentorial or cerebellar herniation. With large hemorrhages, CSF is almost always bloody (Hct > 1%) and under increased pressure.

Treatment is similar to that for ischemic stroke, except that thrombolytics, anticoagulants, and antiplatelet drugs are contraindicated. A narcotic may be needed to relieve headache, and a benzodiazepine to relieve anxiety. Nausea or vomiting may require IV fluids and prochlorperazine 2.5 to 5 mg during the first few days.

Surgical evacuation of large hemorrhages causing brain displacement is often lifesaving in patients with cerebellar hemisphere hematomas > 3 cm in diameter. Early evacuation of polar cerebral hematomas may also be lifesaving, although rebleeding occurs frequently in elderly patients with amyloid angiopathy, and neurologic disability may actually be greater. Early evacuation of deep cerebral hematomas is seldom justified because surgical mortality is high and neurologic disability is usually profound. Survivors of acute hemorrhage sometimes recover surprisingly well, because hemorrhage is less destructive to brain tissue than infarction.

SUBARACHNOID HEMORRHAGE

Sudden bleeding into the subarachnoid space.

Overall, head trauma is the most common cause of subarachnoid hemorrhage (see Ch. 175). Spontaneous (primary) subarachnoid hemorrhage usually results from a ruptured congenital intracranial aneurysm. Less commonly, it is due to a mycotic or arteriosclerotic aneurysm, arteriovenous malformation, or hemorrhagic disease. Aneurysmal hemorrhage may occur at any age but is most common in those aged 40 to 65.

Most aneurysms occur along the middle or anterior cerebral arteries or the communicating branches of the circle of Willis. They usually arise from outpouchings at arterial bifurcations, where the muscular coat is poorly developed; arteriosclerosis and hypertension may also play a role.

A secondary increase in intracranial pressure is common after subarachnoid hemorrhage and may last for days or a few weeks. Communicating hydrocephalus commonly results and may contribute to headache or posthemorrhagic obtundation or dementia.

Symptoms and Signs

Before rupture, aneurysms may be asymptomatic, but warning leaks are often associated with minor headaches. If CT or MRI scans and CSF examinations are normal, sudden, severe headaches are not associated with subsequent subarachnoid hemorrhage; instead, they frequently lead to tension or migraine headaches. However, any new-onset headache or changes in character of a headache must be investigated to exclude aneurysmal subarachnoid hemorrhage and an enlarging aneurysm. A few aneurysms produce symptoms by pressing on adjacent structures. Ocular palsies, diplopia, squint, and facial pain indicate pressure on the 3rd, 4th, 5th, or 6th cranial nerves. Visual loss and a bitemporal field defect indicate pressure on the optic chiasm. Pressure on the optic tract produces noncongruent homonymous hemianopia.

When the aneurysm ruptures, headache is usually acute and severe. Patients may present with only a headache or may have varying degrees of neurologic deficits or changes in consciousness. The mixture of escaping blood and CSF irritates the meninges and increases intracranial pressure, producing headache, vomiting, dizziness, and altera-

steep lateral position, which also reveals the relationship of the pulmonary artery to the aorta. Occasionally, a ventricular septal defect or communication between the right ventricle and the aorta can be seen.

Pulmonary artery: Pulmonary angiography is the definitive technique for diagnosing acute pulmonary embolism; intraluminal filling defects or arterial cutoffs are diagnostic. Contrast material is injected into the main pulmonary artery or right ventricular outflow tract, but selective injection into one or both pulmonary arteries may achieve better definition with less contrast material.

Left atrium: Space-occupying lesions (eg, myxomas, clots) are the usual reason for opacification of the left atrium, although echocardiography is the procedure of choice for diagnosing these lesions. Direct injection for opacification of the left atrium may be hazardous in such cases; instead, the levo phase of a pulmonary angiogram (ie, as dye fills the left atrium from the pulmonary veins) can be safely used.

Left ventricle: A 30 to 45° right anterior oblique projection best demonstrates the long axis of the left ventricle and ventricular aneurysms or areas of asynergy of the anterior wall and separates the left atrium from the left ventricle so that mitral regurgitation can be seen. The left anterior oblique projection defines the left ventricular outflow tract and subvalvular aortic areas as well as the motion of the interventricular septum and left ventricular posterior wall. Cineangiography assesses left ventricular volume, wall motion, and performance. After left ventricular mass and volume are determined from single plane or biplane angiocardiograms, end-systolic and end-diastolic volumes and ejection fraction can be calculated.

Aorta: Aortic regurgitation is best seen by injecting contrast material into the ascending aorta in a 60° left anterior oblique or left lateral projection. Coarctation of the aorta, patent ductus arteriosus, and aortic dissection also are diagnosed from aortic angiograms.

Coronary arteries: Indications for coronary angiography include unstable angina (including post-MI angina unresponsive to or incompletely relieved by proper medical therapy); atypical chest pain; valve disease that might be corrected by valve replacement, especially in patients with a history of angina or syncope; and unexplained heart failure, possibly due to a left ventricular aneurysm.

Physiologic Effects and Complications

A transient sense of warmth, especially in the head and face, is universally experienced after injection. Cardiovascular responses include tachycardia, a slight fall in systemic pressure, and a rise in CO. Nausea, vomiting, and coughing are minor side effects. Major complications (eg, cardiac arrest, anaphylactic reactions, shock, convulsions, cyanosis, renal toxicity) are rare. Patients with a high Hct are susceptible to thrombosis; the Hct should be < 65% before angiography is performed. Allergic reactions may include urticaria and conjunctivitis, which usually respond to diphenhydramine 50 mg IV. Bronchospasm, laryngeal edema, and dyspnea are rare reactions, treated with salbutamol or epinephrine. Ventricular arrhythmias are common if the catheter tip contacts the ventricular endocardium, but ventricular fibrillation is rare. Contrast media, all hypertonic, are excreted by the kidneys.

Percutaneous Transluminal Coronary Angioplasty

Percutaneous transluminal coronary angioplasty (PTCA) is indicated for the revascularization of coronary arteries narrowed by atheroma. Immediate PTCA may be superior to and more cost effective than thrombolytic therapy as initial treatment of MI. However, many centers restrict use to patients in whom thrombolytic therapy is contraindicated. MI patients with developing or established cardiogenic shock should be treated with PTCA rather than thrombolytic therapy. PTCA after failed thrombolytic therapy should be reserved for patients with ongoing ischemia or clinical compromise. Finally, elective PTCA may be performed in post-MI patients who have recurrent or provokable angina before hospital discharge.

Procedure

The appropriate coronary ostium is catheterized with a guiding catheter, allowing the passage of a balloon-tipped catheter distally into the coronary artery. The balloon is aligned within the stenosis and then inflated to dilate the vessel. Angiography is repeated at the completion of the procedure to document any changes.

Various anticoagulation regimens are used during and after angioplasty to reduce the incidence of thrombosis at the site of balloon dilation. Ca blockers and nitrates may also reduce coronary spasm.

The incidence of restenosis is highest in the first 6 mo after angioplasty, with rates as high as 35%. Repeat angioplasty is required in most patients with restenosis, with fewer requiring surgical revascularization.

Coronary artery stents are being used with increased frequency to decrease the need for repeat revascularization procedures. In short nonrestenotic lesions with large native coronary arteries, coronary stenting has reduced the need for repeat revascularization in the short term. The use of stents for restenotic lesions, acute MI, long lesions, diffuse disease, and acute occlusions is still under investigation.

Contraindications

Absolute contraindications include significant obstruction of the left main coronary artery without protection by a nonobstructed bypass graft to the left anterior descending or left circumflex arteries and the lack of cardiac surgical support. Relative contraindications include a coagulopathy or hypercoagulable state, diffusely diseased vessels without focal dilatable disease, a single diseased vessel providing all the perfusion to the myocardium, total coronary occlusion, < 50% stenosis, and vessels perfusing nonischemic areas of the myocardium of patients undergoing angioplasty for acute MI.

Complications

Many complications of PTCA are similar to those of angiocardiography (see above), but risk of death, MI, and stroke are greater. Complications unique to PTCA include abrupt coronary artery closure and restenosis. Abrupt closure may occur in up to 4% of patients; it may be secondary to spasm, dissection, or thrombus formation. Treatment consists of drugs (see treatment of these conditions elsewhere in THE MANUAL), stents, or, in the most extreme circumstances, intra-aortic balloon pumps or emergency coronary artery bypass surgery.

199 / ARTERIAL HYPERTENSION

Elevation of systolic and/or diastolic BP, either primary or secondary.

(For a discussion of hypertension in pregnancy, see Ch. 250.)

Prevalence

It is estimated that there are nearly 50 million hypertensives in the USA (systolic BP ≥ 140 mm Hg and/or diastolic ≥ 90 mm Hg, or taking antihypertensive medication). For unknown reasons, the prevalence of hypertension seems to be decreasing in the USA (see Table 199-1). Hypertension occurs more often in black adults (32%) than in white (24%) or Mexican American (23%) adults, and morbidity and mortality are greater in blacks. Diastolic BP increases with age until age 55 or 60.

Prevalence of isolated systolic hypertension (ISH—≥ 140 mm Hg systolic, < 90 mm Hg diastolic) increases with age until at least age 80. If persons with ISH and diastolic hypertension are considered, > 50% of black and white men and > 60% of women over age 65 have hypertension. ISH is more prevalent among women than men in both races. Prevalence data, derived mainly from large screening programs such as the National Health and Nutrition Examination Survey, rely on one or more BP determinations made during one visit. Thus, these percentages are higher than they would be if BP had been measured over time (regression toward the mean). Between 85 and 90% of cases are primary (essential); in 5 or 10%, hypertension

TABLE 199–1. PREVALENCE OF HYPERTENSION IN MEN AND WOMEN IN THE USA

Race and Ethnic Group	Prevalence, % (SE)	Age-Adjusted Prevalence,* % (SE)	Estimated Population, *n* (SE)†
Non-Hispanic blacks	28.4 (1.4)	32.4 (1.1)	5,672 (427)
Men	29.9 (2.0)	34.0 (1.6)	2,664 (209)
Women	27.3 (1.5)	31.0 (1.0)	3,008 (252)
Non-Hispanic whites	24.6 (1.0)	23.3 (0.7)	34,697 (2,746)
Men	25.6 (1.3)	25.4 (1.2)	17,259 (1,642)
Women	23.8 (1.1)	21.0 (0.9)	17,438 (1,334)
Mexican Americans	14.3 (1.3)	22.6 (0.8)	1,143 (124)
Men	14.6 (1.4)	23.2 (1.1)	604 (68)
Women	14.0 (1.3)	21.6 (1.0)	539 (66)
Overall‡	24.0 (0.9)	24.2 (0.6)	43,186 (2,427)
Men‡	24.7 (1.2)	25.9 (1.0)	21,287 (1,490)
Women‡	23.4 (0.9)	22.2 (0.8)	21,900 (1,238)

*Age adjusted to the 1990 civilian, noninstitutionalized population.
†In thousands.
‡Includes race and ethnic groups not shown separately.
SE = standard error.
Adapted from Burt VL, et al: "Prevalence of hypertension in the US adult population: results from the Third National Health and Nutrition Examination Survey 1988–1991." *Hypertension* 25(3):305–313, 1995.

is secondary to bilateral renal parenchymal disease, and only 1 or 2% of cases are due to a potentially curable condition.

Etiology and Pathogenesis

Primary hypertension: Primary (essential) hypertension is of unknown etiology; its diverse hemodynamic and pathophysiologic derangements are unlikely to result from a single cause. Heredity is a predisposing factor, but the exact mechanism is unclear. Environmental factors (eg, dietary Na, obesity, stress) seem to act only in genetically susceptible persons. Isolated, perfused kidneys from Dahl salt-sensitive rats (which are genetically prone to hypertension when fed a high-salt diet) do not excrete water or Na as rapidly as those from Dahl salt-resistant rats, even before hypertension develops.

The pathogenic mechanisms must lead to increased total peripheral vascular resistance (TPR) by inducing vasoconstriction, to increased cardiac output (CO), or to both because BP equals CO (flow) times resistance. Although expansion of intravascular and extravascular fluid volume is widely claimed to be important, such expansion can only raise BP by increasing CO (by increasing venous return to the heart), by increasing TPR (by causing vasoconstriction), or by both; it frequently does neither.

Abnormal Na transport across the cell wall due to a defect in or inhibition of the Na-K pump (Na^+,K^+-ATPase) or due to increased permeability to Na^+ has been described in some cases of hypertension. The net result is increased intracellular Na, which makes the cell more sensitive to sympathetic stimulation. Because Ca follows Na, it is postulated that the accumulation of intracellular Ca (and not Na per se) is responsible for the increased sensitivity. Na^+,K^+-ATPase may also be responsible for pumping norepinephrine back into the sympathetic neurons to inactivate this neurotransmitter. Thus, inhibition of this mechanism could conceivably enhance the effect of norepinephrine. Defects in Na transport have been described in normotensive children of hypertensive parents.

Stimulation of the sympathetic nervous system raises BP, usually more in hypertensive or prehypertensive patients than in normotensive patients. Whether this hyperresponsiveness resides in the sympathetic nervous system itself or in the myocardium and vascular smooth muscle that it innervates is unknown, but it can often be detected before sustained hypertension develops. A high resting pulse rate, which can be a manifestation of increased sympathetic nervous activity, is a well-known predictor of subsequent hypertension. Some hypertensive patients have a higher-than-normal circulating plasma catecholamine level at rest, especially early in clinical development.

Drugs that depress sympathetic nervous activity frequently reduce BP in patients with primary hypertension. However, this observation cannot be considered evidence for implicating the sympathetic nervous system as the causative factor in primary hypertension. In hypertensive patients, the baroreflexes tend to sustain rather than counteract hypertension, a phenomenon known as "resetting the barostats," which may be a result rather than a cause of hypertension. Some hypertensive patients have defective storage of norepinephrine, thus permitting more to circulate.

In the **renin-angiotensin-aldosterone system,** the juxtaglomerular apparatus helps regulate volume and pressure. Renin, a proteolytic enzyme formed in the granules of the juxtaglomerular apparatus cells, catalyzes conversion of the protein angiotensinogen to angiotensin I, a decapeptide. This inactive product is cleaved by a converting enzyme, mainly in the lung but also in the kidney and brain, to an octapeptide, angiotensin II, which is a potent vasoconstrictor that also stimulates release of aldosterone. Also found in the circulation, the des-ASP heptapeptide (angiotensin III) is as active as angiotensin II in stimulating aldosterone release but has much less pressor activity.

Renin secretion is controlled by at least four mechanisms that are not mutually exclusive: A renal vascular receptor responds to changes in tension in the afferent arteriolar wall; a macula densa receptor detects changes in the delivery rate or concentration of NaCl in the distal tubule; circulating angiotensin has a negative feedback effect on renin secretion; and the sympathetic nervous system stimulates renin secretion via the renal nerve mediated by β receptors.

Plasma renin activity (PRA) is usually normal in patients with primary hypertension but is suppressed in about 25% and elevated in about 15%. Hypertension is more likely to be accompanied by low renin levels in blacks and the elderly. The accelerated (malignant) phase of hypertension is usually accompanied by elevated PRA (see MALIGNANT HYPERTENSIVE ARTERIOLAR NEPHROSCLEROSIS in Ch. 228). Although angiotensin is generally acknowledged to be responsible for renovascular hypertension (see below), at least in the early phase, there is no consensus regarding the role of the renin-angiotensin-aldosterone system in patients with primary hypertension, even in those with high PRA.

The mosaic theory states that multiple factors sustain elevated BP even though an aberration of only one was initially responsible; eg, the interaction between the sympathetic nervous system and the renin-angiotensin-aldosterone system. Sympathetic innervation of the juxtaglomerular apparatus in the kidney releases renin; angiotensin stimulates autonomic centers in the brain to increase sympathetic discharge. Angiotensin also stimulates production of aldosterone, which leads to Na retention; excessive intracellular Na enhances the reactivity of vascular smooth muscle to sympathetic stimulation.

Hypertension leads to more hypertension. Other mechanisms become involved when hypertension due to an identifiable cause (eg, catecholamine release from a pheochromocytoma, renin and angiotensin from renal artery stenosis, aldosterone from an adrenal cortical adenoma) has existed for some time. Smooth muscle cell hypertrophy and hyperplasia in the arterioles resulting from prolonged hypertension reduce the caliber of the lumen, thus increasing TPR. In addition, trivial shortening of hypertrophied smooth muscle in the thickened wall of an arteriole will reduce the radius of an already narrowed lumen to a much greater extent than if the muscle and lumen were normal. This may be why the longer hypertension has existed, the less likely surgery for secondary causes will restore BP to normal.

Deficiency of a vasodilator substance rather than excess of a vasoconstrictor (eg, angiotensin, norepinephrine) may cause hypertension. The kallikrein system, which produces the potent vasodilator bradykinin, is beginning to be studied. Extracts of renal medulla contain vasodilators, including a neutral lipid and a prostaglandin; absence of these vasodilators due to renal parenchymal disease or bilateral nephrectomy would permit BP to rise. Modest hypertension sensitive to Na and water balance is characteristic in anephric persons (renoprival hypertension).

Endothelial cells produce potent vasodilators (nitric oxide, prostacyclin) and the most potent vasoconstrictor, endothelin. Therefore, dysfunction of the endothelium could have a profound effect on BP. The endothelium's role in hypertension is being investigated. Evidence that hypertensive persons have decreased activity of nitric oxide is preliminary.

Secondary hypertension: Secondary hypertension is associated with renal parenchymal disease (eg, chronic glomerulonephritis or pyelonephritis, polycystic renal disease, collagen disease of the kidney, obstructive uropathy) or pheochromocytoma, Cushing's syndrome, primary aldosteronism, hyperthyroidism, myxedema, coarctation of the aorta, or renovascular disease (see RENOVASCULAR HYPERTENSION, below). It may also be associated with the use of excessive alcohol, oral contraceptives, sympathomimetics, corticosteroids, cocaine, or licorice.

Hypertension associated with chronic renal parenchymal disease results from combination of a renin-dependent mechanism and a volume-dependent mechanism. In most cases, increased renin activity cannot be demonstrated in peripheral blood, and meticulous attention to fluid balance usually controls BP.

Diagnosis and treatment of secondary causes of hypertension are dealt with elsewhere in THE MANUAL. The remainder of this discussion focuses almost entirely on primary hypertension.

Pathology

No early pathologic changes occur in primary hypertension. Ultimately, generalized arteriolar sclerosis develops; it is particularly apparent in the kidney (nephrosclerosis) and is characterized by medial hypertrophy and hyalinization. Nephrosclerosis is the hallmark of primary hypertension. Left ventricular hypertrophy and, eventually, dilation develop gradually. Coronary, cerebral, aortic, renal, and peripheral atherosclerosis are more common and more severe in hypertensives because hypertension accelerates atherogenesis. Hypertension is a more important risk factor for stroke than for atherosclerotic heart disease. Tiny Charcot-Bouchard aneurysms, frequently found in perforating arteries (especially in the basal ganglia) of hypertensives, may be the source of intracerebral hemorrhage.

Hemodynamics

Not all patients with primary hypertension have normal CO and increased TPR. CO is increased, and TPR is inappropriately normal for the level of CO in the early labile phase of primary hypertension. TPR increases and CO later returns to normal, probably because of autoregulation. Patients with high, fixed diastolic pressures often have decreased CO. The role of the large veins in the pathophysiology of primary hypertension has largely been ignored, but venoconstriction early in the disease may contribute to the increased CO.

Plasma volume tends to decrease as BP increases, although some patients have expanded plasma volumes. Hemodynamic, plasma volume, and PRA variations are evidence that primary hypertension is more than a single entity or that different mechanisms are involved in different stages of the disorder.

Renal blood flow gradually decreases as the diastolic BP increases and arteriolar sclerosis begins. GFR remains normal until late in the disease, and, as a result, the filtration fraction is increased. Coronary, cerebral, and muscle blood flow are maintained unless concomitant severe atherosclerosis is present in these vascular beds.

In the absence of heart failure, CO is normal or increased, and peripheral resistance is usually high in hypertension due to pheochromocytoma, primary aldosteronism, renal artery disease, and renal parenchymal disease. Plasma volume tends to be high in hypertension due to primary aldosteronism or renal parenchymal disease and may be subnormal in pheochromocytoma.

Systolic hypertension (with normal diastolic pressure) is not a discrete entity. It often results from increased CO or stroke volume (eg, labile phase of primary hypertension, thyrotoxicosis, arteriovenous fistula, aortic regurgitation); in elderly persons with normal or low CO, it usually reflects inelasticity of the aorta and its major branches (arteriosclerotic hypertension).

Symptoms and Signs

Primary hypertension is asymptomatic until complications develop in target organs (eg, left ventricular failure, atherosclerotic heart disease, cerebrovascular insufficiency with or without stroke, renal failure). However, the symptoms of hypertensive encephalopathy due to severe hypertension and cerebral edema are discussed below. Dizziness, flushed facies, headache, fatigue, epistaxis, and nervousness are not caused by uncomplicated hypertension.

A fourth heart sound and broad, notched P-wave abnormalities on the ECG are among the earliest signs of hypertensive heart disease. Echocardiographic evidence of left ventricular hypertrophy may appear later. Chest x-ray is often normal until the late dilated phase of hypertensive heart disease. Aortic dissection or leaking aneurysm of the aorta may be the first sign of hypertension or may complicate untreated hypertension. Polyuria, nocturia, diminished renal concentrating ability, proteinuria, microhematuria, cylindruria, and nitrogen retention are late manifestations of arteriolar nephrosclerosis.

Retinal changes may include retinal hemorrhages, exudates, papilledema, and vascular accidents. On the basis of retinal changes, Keith, Wagener, and Barker classified hypertension into groups that have important prognostic implications: group 1—constriction of retinal arterioles only; group 2—constriction and sclerosis of retinal arterioles; group 3—hemorrhages and exudates in addition to vascular changes; group 4 (malignant hypertension)—papilledema.

Diagnosis

Diagnosis of primary hypertension depends on repeatedly demonstrating higher-than-normal systolic and/or diastolic BP and excluding secondary causes.

At least two BP determinations should be taken on each of 3 days before a patient is diagnosed as hypertensive (see TABLE 199–2). More BP determinations are desirable for patients in the low hypertension range and especially for patients with markedly labile BP. Normal BP is much lower for infants and children (see SCREENING in Ch. 256). Sporadic higher levels in patients who have been resting for > 5 min suggest an unusual lability of BP that may precede sustained hypertension. For example, office or white coat hypertension refers to BP that is consistently elevated in the physician's office but normal when measured at home or by ambulatory BP monitoring.

The basic or minimal evaluation recommended for patients with hypertension includes history and physical examination, CBC, urinalysis, serum analysis (creatinine; K; Na; glucose; total, high density, and low density lipoprotein cholesterol), and ECG. The more severe the hypertension and the younger the patient, the more extensive the evaluation should be. Ambulatory BP monitoring, renal scintigraphy, chest x-ray, screening tests for pheochromocytoma, and renin-sodium profiling are not routinely necessary. Peripheral plasma renin activity has not been helpful in diagnosis or drug selection, but it may be an independent risk factor for coronary disease (but not for stroke or total cardiovascular mortality).

TABLE 199–2. CLASSIFICATION OF BLOOD PRESSURE FOR ADULTS*

Classification	Systolic (mm Hg)		Diastolic (mm Hg)
Optimal	< 120	and	< 80
Normal	< 130	and	< 85
High-normal	130–139	or	85–89
Stage 1 hypertension (mild)	140–159	or	90–99
Stage 2 hypertension (moderate)	160–179	or	100–109
Stage 3 hypertension (severe)	≥ 180	or	≥ 110

*Hypertension classification is based on the average of two or more readings taken at each of two or more visits after initial screening.

Adapted from the Joint National Committee on the Detection, Evaluation, and Treatment of High Blood Pressure. "The sixth report of the Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure (JNC VI)." *Archives of Internal Medicine* 157:2413–2446, 1997.

Pheochromocytoma (see also Ch. 9) secretes catecholamines, which, besides elevating BP, usually produce symptoms (various combinations of headache, palpitations, tachycardia, excessive perspiration, tremor, and pallor) that should alert the physician to this possibility. Catecholamines (eg, epinephrine, norepinephrine) are eventually metabolized in the body to a common product, 3-methoxy-4-hydroxymandelic acid, often called vanillylmandelic acid (VMA). Diagnosis depends on demonstrating increased urinary or plasma concentrations of catecholamine or increased urinary concentrations of metanephrines and VMA.

Hypokalemia not due to diuretics should suggest **primary aldosteronism.** Proteinuria, cylindruria, or microhematuria with or without nitrogen retention early in the course of hypertension is strong evidence of underlying **primary renal disease.** Absent or markedly reduced and delayed femoral arterial pulses in a hypertensive patient aged < 30 yr are presumptive evidence of **coarctation of the aorta.** Cushing's syndrome, collagen disease, toxemia of pregnancy, acute porphyria, hyperthyroidism, myxedema, acromegaly, some CNS disorders, and primary aldosteronism must be excluded; these disorders are discussed elsewhere in THE MANUAL.

Prognosis

An untreated hypertensive patient is at great risk of disabling or fatal left ventricular failure, MI, cerebral hemorrhage or infarction, or renal failure at an early age. Hypertension is the most important risk factor predisposing to stroke. It is one of three risk factors (along with cigarette smoking and hypercholesterolemia) predisposing to coronary atherosclerosis. The higher the BP and the more severe the changes in the retina, the worse the prognosis. Fewer than 5% of patients with group 4 or malignant hypertension characterized by papilledema and < 10% of patients with group 3 changes in the fundus survive 1 yr without treatment. Effective medical control of hypertension will prevent or forestall most complications and will prolong life in patients with ISH or diastolic hypertension. Coronary artery disease is the most common cause of death among treated hypertensive patients. Systolic BP is a more important predictor of fatal and nonfatal cardiovascular events than diastolic BP. In a follow-up of men screened for the Multiple Risk Factor Intervention Trial, overall mortality was related to systolic BP, regardless of diastolic BP.

Treatment

Primary hypertension has no cure, but treatment can modify its course. It is estimated that only 24% of hypertensive patients in the USA have their BP controlled to < 140/90 mm Hg, and 30% are unaware that they have hypertension.

Lifestyle modifications: Extra rest, prolonged vacations, moderate weight reduction, and dietary Na restriction are not as effective as antihypertensive drug therapy. Patients with uncomplicated hypertension need not restrict their activities as long as their BP is controlled. Dietary restrictions can help control diabetes mellitus, obesity, and blood lipid abnormalities. In stage 1 hypertension, weight reduction to ideal levels, modest dietary Na restriction to < 2 g/day, and alcohol consumption to < 1 oz/day may make drug therapy unnecessary. Prudent exercise should be encouraged. Smoking should be unambiguously discouraged.

Antihypertensive drug therapy: Most authorities would agree that patients with systolic BP averaging 140 to 159 mm Hg and/or diastolic BP of 90 to 94 mm Hg should receive antihypertensive drugs if lifestyle modifications do not normalize BP. The benefit of drug therapy for patients with stage 1 hypertension is unequivocal. There are no data on the efficacy of antihypertensive therapy for borderline hypertension. When target organ damage or other risk factors are present, or when the systolic BP is ≥ 160 mm Hg and/or diastolic BP is ≥ 100 mm Hg, drug therapy should not be deferred to await the uncertain results of lifestyle modifications. Heart failure, symptomatic coronary atherosclerosis, cerebrovascular disease, and renal failure require urgent and judicious antihypertensive therapy.

The Systolic Hypertension in the Elderly Trial showed marked benefit from antihypertensive treatment. In patients ≥ 60 yr with systolic BP ≥ 160 and diastolic BP < 90 mm Hg, chlorthalidone (plus atenolol, if necessary) reduced the incidence of stroke (by 36%) and other major cardiovascular events. Benefit was found in both young elderly and old elderly. The goal was to lower systolic BP to < 160 mm Hg and by at least 20 mm Hg for patients whose pretreatment systolic BP was 160 to 179 mm Hg.

Except in patients > 65 yr, the goal of therapy should be to reduce BP to < 135/80 mm Hg or as near to this level as tolerable. Retrospective studies indicate that coronary mortality may increase if diastolic BP is reduced to < 85 mm Hg, especially for patients with clinical evidence of preexisting atherosclerotic heart disease (the so-called J curve). However, other observations have failed to confirm this, and most reports have failed to show a J curve for systolic BP, even when a J curve in diastolic BP was observed. Usually, it is advantageous to have the patient measure BP at home, provided that the patient or a family member is thoroughly instructed and closely monitored and the sphygmomanometer is carefully calibrated at regular intervals.

Drug therapy should be initiated with a diuretic or a β-blocker, unless these drugs are contraindicated or another class of drugs is indicated. If these drugs are ineffective, alternative classes suitable for initial therapy include Ca blockers, ACE inhibitors, angiotensin II receptor blockers, α_1-adrenergic blockers, and α–β-blockers (see TABLE 199–3). However, none of these except nitrendipine, a dihydropyridine Ca blocker, has been shown to reduce cardiovascular morbidity and mortality in prospective, randomized trials, whereas diuretics or β-blockers as initial therapy have shown beneficial effects on cardiovascular and cerebrovascular morbidity and mortality. Nitrendipine significantly reduced fatal and nonfatal strokes but not coronary events in elderly patients with isolated systolic hypertension.

Selection of the initial drug should be guided by age and race of the patient and by coexisting diseases or conditions that may represent a contraindication for certain drugs (eg, asthma and β-blockers) or a special indication for certain drugs (eg, angina pectoris and β-blockers or Ca blockers). In the Veterans Administration Trial of single drug therapy for hypertension in men, black patients responded best to a Ca blocker (diltiazem). Hydrochlorothiazide was more effective in white or black men aged > 60 yr than in younger patients. The β-blocker atenolol was more effective in white patients than in blacks, regardless of age. Race and age are only guidelines to which there are many exceptions.

TABLE 199–3. INITIAL THERAPY WITH ANTIHYPERTENSIVE DRUGS

Drugs	Indications or Patient Characteristics
Diuretics*	Old age Black race Obesity Congestive heart failure Chronic renal failure (loop diuretics)
β-Blockers*	Youth White race Hyperkinetic circulation Angina pectoris Post-MI (cardioprotective effect)*† Migraine headaches† Senile tremor Atrial fibrillation (to control ventricular rate)† Paroxysmal supraventricular tachycardia†
Long-acting Ca blockers	Old age Black race Angina pectoris Atrial fibrillation to control ventricular rate (verapamil and diltiazem) Paroxysmal supraventricular tachycardia (verapamil and diltiazem)
Long-acting Ca blockers *(continued)*	Migraine headaches (verapamil and diltiazem) Isolated systolic hypertension in elderly patients (dihydropyridines)*
ACE inhibitors	Contraindicated in pregnancy Youth White race Left ventricular failure due to systolic dysfunction* Type I diabetes with nephropathy* Heavy proteinuria in chronic renal disease and diabetic glomerulosclerosis Impotence from other drugs
Angiotensin II receptor blockers	Contraindicated in pregnancy Youth White race Conditions for which ACE inhibitors are indicated but cause cough
α-Adrenergic blockers	Prostatism Diabetes mellitus Dyslipidemia

*Reduced morbidity and mortality reported in randomized trials.
†β-Blockers without intrinsic sympathomimetic activity.
Adapted from Gifford RW Jr: "Mild hypertension: Critical analysis of different therapeutic approaches." *Cleveland Clinic Journal of Medicine* 56:337–345, 1989.

f the initial drug is ineffective or causes olerable adverse effects, another can be ostituted (sequential monotherapy). Alterively, if the original drug is only partially ective but well tolerated, the dose may be reased or a second drug can be added, uch should be of a different class (stepped re). The central-acting sympathetic inhibng drugs are not recommended for initial erapy because of their high adverse effect ofile. However, they are effective and can used in small doses in combination regiens. A direct vasodilator (hydralazine or inoxidil) may be used with a diuretic to revent fluid retention and with a β-blocker o prevent reflex tachycardia.

Pr[illegible]ably, treatment is started with only one [illegible]g unless hypertension is severe. However, combinations of a diuretic with a β-blocker or an ACE inhibitor are available in single tablets in subtherapeutic doses of each compound that together have an antihypertensive effect with minimal adverse effects. Two of these combinations are available in the USA for initial therapy of stage 1 or 2 hypertension (see TABLE 199–4). Three or four drugs in combination may be necessary for severe or resistant hypertension.

TABLE 199–4. COMBINATION DRUGS USED FOR ARTERIAL HYPERTENSION

Diuretic with β-blocker	Propanolol hydrochloride and hydrochlorothiazide*
	Metoprolol tartrate and hydrochlorothiazide*
	Atenolol and chlorthalidone*
	Timolol maleate and hydrochlorothiazide*
	Bisoprolol fumarate and hydrochlorothiazide†
Diuretic with [illegible]E inhibitor	Captopril and hydrochlorothiazide†
	Benazepril hydrochloride and hydrochlorothiazide*
	Lisinopril and hydrochlorothiazide*
	Enalapril maleate and hydrochlorothiazide*
ACE inhibitor with Ca blocker	Amlodipine and benazepril hydrochloride*
Angiotensin II receptor blocker with diuretic	Losartan and hydrochlorothiazide*

*Not approved for initial therapy.
†Approved for initial therapy.

All thiazide derivatives and their congeners are equally effective in equivalent doses (see TABLE 199–5). Metolazone, indapamide, and the loop diuretics furosemide, bumetanide, ethacrynic acid, and torsemide are no more effective than the thiazides but are preferred in patients with chronic renal failure. The antihypertensive action of diuretics seems to be due to a modest reduction in plasma volume and a decrease in vascular reactivity, possibly mediated by shifts in Na from intracellular to extracellular loci.

K supplementation or the use of a K-sparing diuretic is recommended with kaliuretic diuretics for patients who are also taking digitalis, have known heart disease, have an abnormal ECG, have ectopy or arrhythmias, or develop ectopy or arrhythmias while taking the diuretic. The K-sparing distal tubular diuretics (spironolactone, triamterene, amiloride) do not cause hypokalemia, hyperuricemia, or hyperglycemia, but they are not as effective as the thiazides in controlling hypertension. Instead of K supplementation, spironolactone 25 to 100 mg/day, triamterene 50 to 150 mg/day, or amiloride 5 to 10 mg/day can be added to thiazide therapy to treat or prevent hypokalemia.

A disadvantage of diuretics is sexual dysfunction, which occurs more commonly than with some of the other drugs proposed for initial therapy. Metabolic adverse effects of diuretics (hypokalemia, hypomagnesemia, hyperuricemia, hyperglycemia, hypercalcemia, hyperlipidemia) are dose-related and, if properly managed, do not usually prevent diuretic use. Spironolactone can cause breast tenderness, making amiloride or triamterene preferable when a K-sparing drug is chosen for males.

Diuretics uncommonly precipitate clinical type II diabetes or aggravate preexisting type II diabetes in susceptible patients. Most diabetics can tolerate a low-dose thiazide diuretic with little or no effect on the control of their diabetes, although it may aggravate hyperinsulinemia. Exercise and weight loss will ameliorate but not eliminate these adverse effects.

Thiazide and related diuretics can increase serum cholesterol (mostly in the low-density lipoprotein fraction) and triglyceride

Class	Drug	Trade Name	Usual Daily Dose	Selected Adverse Effects	Comments
Thiazide and related diuretics	Bendroflumethiazide	Naturetin	2.5–5 mg	Hypokalemia, hyperuricemia, glucose intolerance, hypercholesterolemia, hypertriglyceridemia, hypercalcemia, sexual dysfunction in men, weakness, rash	Except for indapamide and metolazone; may be ineffective in renal failure; hypokalemia increases digitalis toxicity; may increase blood levels of lithium
	Chlorothiazide	Diuril	125–500 mg		
	Chlorthalidone	Hygroton	12.5–50 mg		
		Thalitone	15–50 mg		
	Hydrochlorothiazide	HydroDIURIL	12.5–50 mg		
		Esidrix	12.5–50 mg		
		Microzide	12.5–50 mg		
		Oretic	12.5–50 mg		
	Hydroflumethiazide	Diucardin	25–50 mg		
	Indapamide	Lozol	2.5–5 mg		
	Methyclothiazide	Enduron	2.5–5 mg		
	Metolazone	Zaroxolyn	2.5–10 mg		
		Mykrox	0.5–1 mg		
Loop diuretics*	Bumetanide	Bumex	0.5–5 mg†	Same as for thiazide and related diuretics (except for hypercalcemia)	Same as for thiazide and related diuretics except effective in chronic renal failure
	Ethacrynic acid	Edecrin	25–100 mg†		
	Furosemide	Lasix	20–320 mg†		
	Torsemide	Demadex	5–20 mg		
K-sparing diuretics	Amiloride	Midamor	5–20 mg	Hyperkalemia; nausea, GI distress, gynecomastia, and menstrual irregularities (spironolactone)	Hyperkalemia in patients with renal failure or in patients treated with an ACE inhibitor, an angiotensin II receptor blocker, or an NSAID; may increase blood levels of lithium
	Spironolactone	Aldactone	25–100 mg		
	Triamterene	Dyrenium	100–300 mg		
Combinations of thiazide and K-sparing diuretics	Hydrochlorothiazide/spironolactone 25/25 mg	Aldactazide-25	1–2 tablets/day	See above	See above
	Hydrochlorothiazide/spironolactone 50/50 mg	Aldactazide-50	1 tablet/day		
	Hydrochlorothiazide/triamterene 25/37.5 mg	Dyazide	1–2 capsules/day		

Table continues on the following page.

TABLE 199–5. ORAL DIURETICS USED FOR ARTERIAL HYPERTENSION (*Continued*)

Class	Drug	Trade Name	Usual Daily Dose	Selected Adverse Effects	Comments
Combinations of thiazide and K-sparing diuretics *(continued)*	Hydrochlorothiazide/triamterene 25/37.5 mg	Maxzide-25	0.5–2 tablets/day		
	Hydrochlorothiazide/triamterene 50/75 mg	Maxzide	0.5–1 tablet/day		
	Hydrochlorothiazide/amiloride 50/5 mg	Moduretic	0.5–1 tablet/day		

*Larger doses may be required in patients with renal failure.
†Usually given in divided doses twice per day.

concentration, although most long-term studies failed to show an adverse effect at > 1 yr. Furthermore, increased concentration seems to occur only in susceptible patients, is apparent within 4 wk of treatment, and can be ameliorated by a low-fat diet. Elevated concentration of serum cholesterol or triglycerides is not an a priori contraindication to the use of diuretics in the management of hypertension, because the lipidemic effect is more likely to occur in patients with normal concentrations than in patients with hyperlipidemia.

A hereditary predisposition probably explains the few cases in which diuretic-induced hyperuricemia has led to clinical gout. The Hypertension Detection and Follow-Up Program recorded only 15 cases of gout in 5 among 3693 participants at risk. Diuretic-induced hyperuricemia in the absence of gout is not an indication for antiuricemic therapy, nor does it contraindicate continued diuretic use. Diuretics are less expensive than the alternatives for initial therapy.

All β-blockers (see TABLE 199–6) are equivalent in terms of antihypertensive efficacy. If the patient also has diabetes mellitus, chronic occlusive peripheral arterial disease, or COPD, it is preferable to use a cardioselective β-blocker (acebutolol, atenolol, betaxolol, bisoprolol, metoprolol). However, cardioselectivity is only relative and diminishes as the dose of the β-blocker increases. Even cardioselective β-blockers are contraindicated in the presence of severe asthma or COPD with a prominent bronchospastic component. Use of a cardioselective β-blocker in the absence of one of these indications offers no advantage over nonselective β-blockers.

β-Blockers with intrinsic sympathomimetic activity (ISA—eg, acebutolol, carteolol, penbutolol, pindolol) do not have an adverse effect on serum lipids; they are also less likely to produce severe bradycardia than are non-ISA β-blockers. However, asymptomatic sinus bradycardia, even with rates in the 40s, usually is not harmful.

β-Blockers without ISA and without α-blocking properties have a cardioprotective effect for patients who have had an MI; these drugs are thus indicated for such hypertensive patients.

Disadvantages of β-blockers include a high incidence of CNS adverse effects (sleep disturbances, fatigue, lethargy) and contraindications (greater than first-degree heart block, asthma, sick sinus syndrome, heart failure). Similar to diuretics, β-blockers can cause sexual dysfunction in men and metabolic adverse effects, including impaired glucose tolerance, depressed high density lipoprotein cholesterol, and increased serum total cholesterol and triglyceride concentrations.

Similar to ISA β-blockers, the α–β-blocker labetalol does not reduce resting pulse rate as much as the non-ISA β-blockers and does not seem to have an adverse effect on serum lipids.

Ca blockers (see TABLE 199–7) are potent peripheral vasodilators and reduce BP by decreasing TPR. The diphenylalkylamine derivative verapamil and the benzothiazepine derivative diltiazem slow the heart rate, decrease atrioventricular conduction, and have a negative inotropic effect on myocardial contractility, similar to β-blockers. Consequently, they should not be prescribed for patients with greater than first-degree heart block or left ventricular failure. In general, β-blockers and verapamil or diltiazem should not be prescribed in the same regimen for patients with left ventricular dysfunction.

The dihydropyridine derivatives (amlodipine, felodipine, isradipine, nicardipine, nifedipine, nisoldipine) have a lesser negative inotropic effect than the nondihydropyridines but can sometimes cause reflexive tachycardia. These drugs are more potent peripheral vasodilators than are the nondihydropyridines and should therefore be more effective. However, in long-term antihypertensive therapy, they do not seem to be more potent than nondihydropyridine Ca blockers.

TABLE 199–6. β-BLOCKERS USED FOR ARTERIAL HYPERTENSION

Drug	Trade Name	Usual Daily Dose	Selected Adverse Effects	Comments
Acebutolol*†	Sectral	200–800 mg	Bronchospasm, fatigue, insomnia, sexual dysfunction, exacerbation of heart failure, masking of symptoms of hypoglycemia, triglyceridemia, decreased high density lipoprotein cholesterol (except for pindolol, acebutolol, penbutolol, carteolol, and labetalol)	Contraindicated in patients with asthma, greater than first-degree heart block, or sick sinus syndrome; use with caution in heart failure and insulin-treated diabetics; should not be discontinued abruptly in patients with ischemic heart disease; carvedilol has been approved for treating congestive heart failure
Atenolol*	Tenormin	25–100 mg		
Betaxolol*	Kerlone	10–20 mg		
Bisoprolol*	Zebeta	2.5–20 mg		
Carteolol†	Cartrol	2.5–10 mg		
Carvedilol‡	Coreg	12.5–50 mg§		
Labetalol‡	Normodyne	200–1800 mg§		
	Trandate	200–1800 mg§		
Metoprolol*	Lopressor	50–300 mg§		
	Toprol XL	50–300 mg		
Nadolol	Corgard	40–240 mg		
Penbutolol†	Levatol	20–40 mg		
Pindolol†	Visken	10–60 mg§		
Propranolol	Inderal	40–320 mg§		
Propranolol, long acting	Inderal LA	60–320 mg		
Timolol	Blocadren	20–60 mg§		

*Cardioselective.
†Partial agonist (intrinsic sympathomimetic) activity.
‡An α–β-blocker. Labetalol can be given IV for hypertensive emergencies.
§Usually given in divided doses twice per day.

Short-acting nifedipine has been associated in nonrandomized case-control and cohort studies with increased rates of MI compared with other classes of drugs and therefore should not be used to treat hypertension (for which it is not indicated). Short-acting diltiazem also is not indicated for treating hypertension. Long-acting Ca blockers are preferred.

A Ca blocker is preferred to a β-blocker for hypertensive patients with angina pectoris who also have bronchospastic disease or Raynaud's disease.

Ca blockers do not have metabolic adverse effects, but they can be more expensive than ACE inhibitors.

ACE inhibitors (see TABLE 199–8) are vasodilators that reduce BP by interfering with the generation of angiotensin II from angiotensin I and by inhibiting the degradation of bradykinin, thereby decreasing peripheral vascular resistance without inciting reflex tachycardia. They reduce BP in many hypertensive patients, regardless of plasma renin activity.

TABLE 199–7. CALCIUM BLOCKERS USED FOR ARTERIAL HYPERTENSION

Class	Drug	Trade Name	Usual Daily Dose	Selected Adverse Effects	Comments
Benzothiazepine derivatives	Diltiazem, sustained release	Cardizem SR	120–360 mg*	Headache, dizziness, asthenia, flushing, edema, negative inotropic effect	Contraindicated in heart failure due to systolic dysfunction, sick sinus syndrome, or greater than first-degree heart block; may cause liver dysfunction
	Diltiazem, extended release	Cardizem CD Dilacor XR Tiazac	120–360 mg 120–360 mg 120–360 mg		
Diphenylalkylamine derivatives	Verapamil	Calan Isoptin	120–360 mg† 120–360 mg†	Same as for benzothiazepine derivatives plus constipation	Same as for benzothiazepine derivatives
	Verapamil, sustained release	Covera-HS Calan SR Isoptin SR Verelan	120–480 mg‡ 120–480 mg 120–480 mg 120–480 mg		
Dihydropyridines	Amlodipine	Norvasc	2.5–10 mg	Dizziness, flushing, headache, weakness, nausea, heartburn, pedal edema, tachycardia	Contraindicated in congestive heart failure with the possible exception of amlodipine; nonrandomized studies have shown an association between therapy with short-acting nifedipine and an increase in MI
	Felodipine	Plendil	5–20 mg		
	Isradipine	DynaCirc DynaCirc CR	5–20 mg* 5–20 mg		
	Nicardipine	Cardene	60–120 mg†		
	Nicardipine, sustained release	Cardene SR	60–120 mg*		
	Nifedipine, extended release	Procardia XL Adalat CC	30–90 mg 30–90 mg		
	Nisoldipine	Sular	10–60 mg		

*Usually given in divided doses twice per day.
†Usually given in divided doses three times per day.
‡Entire dose should be given at bedtime.

TABLE 199–8. ACE INHIBITORS AND ANGIOTENSIN II RECEPTOR BLOCKERS USED FOR ARTERIAL HYPERTENSION

Drug	Trade Name	Usual Daily Dose	Selected Adverse Effects	Comments
ACE inhibitors				
Benazepril	Lotensin	10–40 mg	Rash, cough, angioedema, hyperkalemia, dysgeusia	Contraindicated in pregnancy; can cause reversible acute renal failure in patients with bilateral renal arterial stenosis or unilateral stenosis in a solitary kidney; proteinuria may occur (rare at recommended doses); hyperkalemia can develop, particularly in patients with renal insufficiency or taking NSAIDs, K-sparing diuretics, or K supplements; rarely, can induce neutropenia; hypotension has been observed with initiation of treatment, especially in patients with high plasma renin activity or in those receiving diuretic therapy or with other causes of hypovolemia
Captopril	Capoten	25–300 mg*		
Enalapril	Vasotec	5–40 mg		
Fosinopril	Monopril	10–60 mg		
Lisinopril	Prinivil Zestril	5–40 mg 5–40 mg		
Moexipril	Univasc	7.5–30 mg*		
Quinapril	Accupril	5–80 mg		
Ramipril	Altace	2.5–10 mg		
Trandolapril	Mavik	1–4 mg		
Angiotensin II receptor blockers				
Irbesartan	Avapro	75–300 mg	Dizziness, angioedema (rare)	Contraindicated in pregnancy; with the exception of proteinuria and neutropenia, this class of drugs can theoretically produce the same adverse effects as ACE inhibitors on renal function, serum K, and BP
Losartan	Cozaar	25–100 mg		
Valsartan	Diovan	80–320 mg		

*Usually given in divided doses twice per day.

One of the advantages of ACE inhibitors in the management of hypertension is the low adverse effect profile. A dry irritating cough is the most frequent adverse effect. ACE inhibitors do not adversely affect serum lipids, plasma glucose, or uric acid. They tend to increase serum K^+, especially in patients with chronic renal failure or in patients taking K-sparing diuretics, K supplements, or NSAIDs. These drugs are least likely to cause sexual dysfunction in males. Angioedema is a rare adverse effect of ACE inhibitors and can be life-threatening if it involves the oropharyngeal area.

ACE inhibitors reduce proteinuria for patients with diabetic nephropathy and may retard glomerulosclerosis by selectively dilating the efferent (postglomerular) arteriole, thus reducing glomerular capillary pressure without compromising blood flow. They retard the loss of renal function in patients with nephropathy due to type I diabetes. If ACE inhibitors are prescribed for patients with chronic renal disease, especially when azotemia is present, serum creatinine and K^+ levels should be monitored frequently. ACE inhibitors can cause acute renal failure in patients who have severe bilateral renal artery stenosis or severe stenosis in the artery to a solitary kidney, presumably because under these conditions GFR is maintained by angiotensin II–mediated constriction of the efferent arteriole, which is abolished by ACE inhibition. For the same reason, they can cause acute renal failure in hypovolemic patients and in patients with severe heart fail-

ure. Nevertheless, ACE inhibitors reduce mortality and re-hospitalization rates for patients with left ventricular dysfunction and ejection fractions < 40%.

Diuretics consistently enhance the antihypertensive activity of ACE inhibitors as much as, if not more than, they do for any other class of antihypertensive drugs.

A disadvantage of treatment with ACE inhibitors is expense.

Angiotensin II receptor blockers (see TABLE 199–8) block angiotensin II receptors and therefore interfere with the renin-angiotensin system, perhaps more completely than do the ACE inhibitors. They do not block the degradation of bradykinin, which perhaps explains why they do not cause a dry irritating cough. To the extent that bradykinin may contribute to the hypotensive effect of ACE inhibitors, the angiotensin II receptor blockers may less effectively reduce BP. Studies have shown that they are equally effective as antihypertensive drugs. Angiotensin II receptor blockers seem to be remarkably free of adverse effects and have been implicated in fewer cases of angioedema than have the ACE inhibitors, but this adverse effect is very rare with either class of drugs. Presumably, angiotensin II receptor blockers have the same beneficial effects as ACE inhibitors in patients with left ventricular failure and in type I diabetics with nephropathy, but definitive controlled trials have not been reported. Precautions for the use of ACE inhibitors in patients with renovascular hypertension, hypovolemia, and severe heart failure also apply to the angiotensin II receptor blockers.

Adrenergic inhibitors (see TABLE 199–9) include α_2 agonists, which have a central action and are more likely than other drugs to produce drowsiness, lethargy, and sometimes depression. Methyldopa, clonidine, guanabenz, and guanfacine reduce sympathetic nervous activity by stimulating the presynaptic α_2-adrenergic receptors in the brain stem. Clonidine is available for transdermal administration in 2.5-, 5-, or 7.5-mg impregnated patches applied once weekly, delivering respectively 0.1, 0.2, or 0.3 mg/day. This unique dosage form seems to be as effective as the oral route with fewer adverse effects. However, about 20% of patients develop cutaneous reactions at the site of application, requiring discontinuation of the drug in this form.

Prazosin, terazosin, and doxazosin are **peripheral postsynaptic α_1-adrenergic blockers** that act on veins and arterioles. They all relieve symptoms of benign prostatic hyperplasia and are the only group of antihypertensive drugs that have a modest effect on reducing serum cholesterol, especially the low density lipoprotein fraction.

Guanethidine and guanadrel block sympathetic transmission at the neuroeffector junction and, similar to reserpine, deplete tissue stores of norepinephrine. Guanethidine, in particular, is potent but difficult to titrate, so it has largely been discontinued with the advent of newer drugs. Guanadrel is a shorter-acting drug than guanethidine and produces fewer adverse effects. Reserpine depletes the brain of norepinephrine and serotonin and also depletes the peripheral sympathetic nerve terminals of norepinephrine. Except for α_1 receptor blockers, these adrenergic blockers are not recommended for routine initial therapy because they may cause subtle fluid retention, leading to pseudotolerance, and they also have higher adverse effect profiles than the drugs of choice.

The mechanism of **direct vasodilators** (independent of the autonomic nervous system) is different from that of Ca blockers and ACE inhibitors (see TABLE 199–10): Minoxidil is more potent than hydralazine but is associated with more adverse effects, including Na and water retention and hirsutism, which is poorly tolerated by women; it should be reserved for severe, resistant hypertension. Hydralazine has long been used as (and remains) a step-3 drug because its antihypertensive effect is additive to that of other vasodilating drugs. The lupus syndrome is rarely observed if the dosage is < 300 mg/day.

Vasodilating prostaglandins and compounds that enhance endothelial production of nitric oxide, depress endothelin release, or block endothelin receptors are in development.

Drug treatment of hypertensive emergencies: Hypertensive crises may be classified as true emergencies requiring immediate reduction of BP to prevent new possibilities in treating hypertension.

TABLE 199–9. ADRENERGIC INHIBITORS USED FOR ARTERIAL HYPERTENSION

Class	Drug	Trade Name	Usual Dose	Selected Adverse Effects	Comments
Central-acting α agonists	Clonidine	Catapres	0.1–1.2 mg/day*	Drowsiness, sedation, dry mouth, fatigue, sexual dysfunction; localized skin reaction to clonidine patch	Rebound hypertension may occur with abrupt discontinuance, particularly if prior administration of high doses or continuation of concomitant β-blocker
	Clonidine transdermal	Catapres TTS	0.1–0.3 mg/wk		
	Guanabenz	Wytensin	4–64 mg/day*		
	Guanfacine	Tenex	1–3 mg/day		
	Methyldopa	Aldomet	500–2000 mg/day*		methyldopa may cause liver damage. Coombs'-positive hemolytic anemia, should be used cautiously in elderly tients because of orthostatic hypotel and interferes with measurements o nary catecholamine levels by fluoroi ric methods
α-Adrenergic blockers	Doxazosin	Cardura	1–16 mg/day	"First-dose" syncope, orthostatic hypotension, weakness, palpitations, headache	Use cautiously in elderly patients bec of orthostatic hypotension; relieves toms of benign prostatic hypertroph
	Prazosin	Minipress	2–20 mg/day*		
	Terazosin	Hytrin	1–20 mg/day		
Peripheral-acting adrenergic blockers	Guanadrel sulfate	Hylorel	10–100 mg/day*	Diarrhea, sexual dysfunction, orthostatic hypotension (for guanadrel sulfate and guanethidine); lethargy, nasal congestion, depression, activation of peptic ulcer (for Rauwolfia alkaloids and reserpine)	Contraindicated in patients with histo mental depression (reserpine); use caution in patients with history of p ulcer (reserpine); use cautiously be of orthostatic hypotension (guanad sulfate, guanethidine)
	Guanethidine sulfate	Ismelin	10–100 mg/day		
	Rauwolfia alkaloids	—	50–100 mg/day		
	Reserpine	—	0.1–0.2 mg/day		

*Given in divided doses twice per day.

TABLE 199–10. PARENTERAL DRUGS USED FOR HYPERTENSIVE EMERGENCIES

Drug	Dose	Onset of Action	Duration of Action	Adverse Effects*	Special Indications
Vasodilators					
Sodium nitroprusside	0.25–1.0 μg/kg/min IV infusion† (maximum dose for 10 min only)	Immediate	1–2 min	Nausea, vomiting, muscle twitching, sweating, thiocyanate and cyanide intoxication	Most hypertensive emergencies; caution with high intracranial pressure or azotemia
Nicardipine hydrochloride	5–15 mg/h IV	5–10 min	1–4 h	Tachycardia, headache, flushing, local phlebitis	Most hypertensive emergencies, except acute heart failure; caution with coronary ischemia
Fenoldopam mesylate	0.1–0.3 μg/kg/min IV infusion	< 5 min	30 min	Tachycardia, headache, nausea, flushing	Most hypertensive emergencies; caution with coronary ischemia
Nitroglycerin	5–100 μg/min IV infusion†	2–5 min	3–5 min	Headache, vomiting, methemoglobinemia, tolerance with prolonged use	Coronary ischemia
Enalaprilat	1.25–5 mg q 6 h IV	15–30 min	6 h	Precipitous fall in BP in high-renin states; variable response	Acute left ventricular failure; avoid in acute MI
Hydralazine hydrochloride	10–20 mg IV 10–50 mg IM	10–20 min 20–30 min	3–8 h	Tachycardia, flushing, headache, vomiting, aggravation of angina	Eclampsia
Diazoxide	50–100 mg IV bolus repeated or 15–30 mg/min IV infusion	2–4 min	6–12 h	Nausea, flushing, tachycardia, chest pain	Now obsolete when no intensive monitoring available
Adrenergic inhibitors					
Labetalol hydrochloride	20–80 mg IV bolus q 10 min or 0.5–2 mg/min IV infusion	5–10 min	3–6 h	Vomiting, scalp tingling, burning in throat, dizziness, nausea, heart block, orthostatic hypotension	Most hypertensive emergencies, except acute heart failure
Esmolol hydrochloride	250–500 μg/kg/min for 1 min, then 50–100 μg/kg/min for 4 min; may repeat sequence	1–2 min	10–20 min	Hypotension, nausea	Aortic dissection, perioperative period
Phentolamine	5–15 mg IV	1–2 min	3–10 min	Tachycardia, flushing, headache	Catecholamine excess

*Hypotension may occur with all drugs.
†Requires a special delivery system.

ate reduction of BP (eg, hypertensive encephalopathy, acute left ventricular failure with pulmonary edema, eclampsia, acute aortic dissection, severe hypertension accompanying unstable angina or acute MI), usually with parenteral drugs (see TABLE 199–11), or hypertensive urgencies in which the physician is more concerned than the patient. Hypertensive urgencies are frequently overtreated.

Prompt BP reduction with parenteral drugs is indicated for patients with hypertensive encephalopathy, acute left ventricular failure, or other true emergencies. IV diazoxide, sodium nitroprusside, nitroglycerin, nicardipine, or labetalol is usually used for this purpose. Because diazoxide is a nondiuretic thiazide derivative that can cause fluid retention, furosemide 40 or 80 mg IV is usually given with it. Diazoxide is administered by rapid IV injections of 50 to 100 mg (1 to 1.5 mg/kg, ≤ 100 mg/dose) given q 5 to 10 min until the BP reaches the optimal level. Adverse effects include nausea, vomiting, hyperglycemia, hyperuricemia, tachycardia, and, only occasionally, hypotension (generally without shock).

Sodium nitroprusside 0.25 to 10 μg/kg/min (for ≤ 10 min at the highest dose to minimize the risk of cyanide toxicity) given by continuous IV infusion in 5% D/W can promptly reduce BP in a hypertensive crisis, but its evanescent effect and potency require almost continuous monitoring of BP in an ICU. Unlike diazoxide, it produces venodilation and arteriolar dilation and therefore reduces preload and afterload, making it especially useful for managing hypertensive patients with heart failure. Adverse effects include nausea, vomiting, agitation, muscu-

TABLE 199–11. VASODILATORS USED FOR ARTERIAL HYPERTENSION

Drug	Trade Name	Usual Daily Dose	Selected Adverse Effects	Comments
Vasodilators (general)			Headache, tachycardia, fluid retention	May precipitate angina pectoris in patients with coronary artery disease
Vasodilators (specific)				
Hydralazine	Apresoline	50–300 mg*	Positive antinuclear antibody test	May cause lupus syndrome (rare at recommended doses)
Minoxidil	Loniten	2.5–80 mg*	Hypertrichosis	May cause or aggravate pleural and pericardial effusions

*Usually given in divided doses twice per day.

lar twitching, and cutis anserina (goose flesh) if BP is reduced too rapidly. Acute psychosis from thiocyanate intoxication can result from prolonged therapy, especially in patients with renal failure. The drug should be discontinued if the serum thiocyanate concentration is > 12 mg/dL (206 μmol/dL).

Nitroglycerin, similar to sodium nitroprusside, relaxes the resistance vessels and the large capacitance veins. Compared with sodium nitroprusside, it has a greater effect on veins than on arterioles. IV infusions of nitroglycerin have been used to manage hypertension during and after coronary bypass, heart failure, acute MI, unstable angina pectoris and acute pulmonary edema. Hemodynamic studies indicate that IV nitroglycerin is preferable to sodium nitroprusside in managing hypertension associated with severe coronary disease because it increases coronary flow, whereas sodium nitroprusside tends to decrease coronary flow to ischemic areas, possibly because of a "steal" mechanism. The most frequent adverse reaction is headache, which occurs in about 2% of patients; tachycardia, nausea, vomiting, apprehension, restlessness, muscular twitching, and palpitations have also been observed.

Labetalol 20 to 40 mg IV q 10 min or as an infusion is as effective as nitroprusside, diazoxide, or nitroglycerin in managing hypertensive crises. Serious hypotensive episodes have not been observed when labetalol is given by this method, and adverse effects have been minimal. Because of its β-blocking activity, labetalol should probably not be used for hypertensive emergencies in patients with acute left ventricular failure or in asthmatic patients.

Although short-acting nifedipine given orally usually reduces BP rapidly, it has been associated with acute cardiovascular and cerebrovascular events (sometimes fatal) and is not recommended for treating hypertensive emergencies or urgencies. It is not indicated for managing hypertension.

RENOVASCULAR HYPERTENSION

Acute or chronic elevation of systemic BP caused by partial or complete occlusion of one or more renal arteries or their branches, often correctable by surgery or percutaneous transluminal angioplasty.

Stenosis or occlusion of one or both main renal arteries or their branches or an accessory renal artery or its branches can cause hypertension by inciting release of the enzyme renin from juxtaglomerular cells of the affected kidney. The area of the lumen must be decreased by ≥ 70% before the stenosis is hemodynamically significant.

In patients > 50 yr old (usually men), the most frequent cause of renal arterial stenosis is atherosclerosis; in younger patients (usually women), it is one of the fibrous dysplasias. Rarer causes of renal arterial stenosis or obstruction include emboli, trauma, inadvertent ligation during surgery, and extrinsic compression of the renal pedicle by tumors.

Although renovascular disease is the most frequent cause of curable hypertension (with the possible exceptions of oral contraceptive therapy in women and excessive alcohol intake), it accounts for < 2% of all cases of hypertension.

Symptoms, Signs, and Diagnosis

Renovascular hypertension should be suspected when diastolic hypertension first develops in a patient < 30 or ≥ 55 yr old or when previously stable hypertension abruptly accelerates. Rapid progression to malignant hypertension within 6 mo of onset suggests renal artery disease. A systolic-diastolic bruit in the epigastrium, usually transmitted to one or both upper quadrants and sometimes through to the back, is an almost pathognomonic physical finding, but unfortunately it is absent in about 50% of patients with fibrous disease and is rarely heard in patients with atherosclerotic renovascular disease. Trauma to the back or flank, or acute pain in this region with or without hematuria, should alert the physician to the possibility of renovascular hypertension, but these historic features are rare. Renovascular hypertension is characterized by high cardiac output and high peripheral resistance.

Renovascular and primary hypertension are usually asymptomatic, and only the history, the presence of an epigastric bruit, or abnormalities on IVU or technetium 99–pentetic acid (^{99}Tc-DTPA) scintiscan will distinguish them. The main justification for diagnostic evaluation is to find a surgically curable lesion.

No available test is ideal. All give false-positive and false-negative results, all are expensive, and some are hazardous. The most widely used screening test, replacing the rapid-sequence IVU, is the ^{99}Tc-DTPA scintiscan. Delayed perfusion or decreased function of one kidney on the ^{99}Tc-DTPA scintiscan suggests ischemia. The sensitivity and specificity can be enhanced by comparing scans done before and after the oral administration of captopril.

Doppler ultrasonography (duplex scan) is a reliable noninvasive method for determining the presence or absence of significant stenosis (eg, > 60%) in the main renal arteries. The sensitivity and specificity of this technique approach 90% in experienced hands. Unfortunately, the presence of > 60% stenosis in one or both renal arteries does not per se indicate that it is the cause of the hypertension, but this finding, combined with the typical clinical scenario, is highly suggestive of renovascular hypertension. Measurements of renal vein renin activity are rarely often necessary and are sometimes misleading in diagnosing renovascular hypertension.

Before intervention is planned (ie, surgery, angioplasty), arteriography should be performed. Digital subtraction or Seldinger arteriography with selective injection of the renal arteries can confirm the diagnosis and can detect branch lesions not identified by Doppler ultrasonography. IV digital subtraction arteriography is not as reliable as the Seldinger technique in identifying orificial or branch lesions. A normal rapid-sequence IVU or ^{99}Tc-DTPA scintiscan or failure to demonstrate significant stenosis by Doppler ultrasonography does not rule out the need for arteriography if other indications warrant it.

Prognosis and Treatment

Without treatment, the prognosis is similar to that in untreated primary hypertension. Most investigators have found that appropriate surgery will relieve hypertension if the renal vein renin activity ratio (involved to uninvolved side) is > 1.5:1. However, many patients with renal vein renin activity ratios less than this have also been cured of hypertension by revascularization or removal of the ischemic kidney. There is evidence that hypertension < 5 yr and appropriate abnormalities on the rapid-sequence IVU or scintigraphy, when considered together, are just as reliable in predicting outcome of surgery as is the renal vein renin activity ratio. To enhance the reliability of the renal vein renin activity ratio, blood should be obtained from the renal veins under conditions of Na depletion to stimulate the release of renin. This can be accomplished by following a 0.5-g Na diet with oral diuretics for 24 h or by injecting furosemide 40 to 80 mg IV and obtaining blood 30 min later. Bilateral lesions, which occur in ≥ 35% of cases, make rapid-sequence IVU, ^{99}Tc-DTPA scintiscan, and the renal vein renin activity ratio less dependable. Unstimulated peripheral vein renin activity is often normal in renovascular hypertension, but a sharp rise in renin activity 60 min after oral administration of captopril 50 mg to ≥ 150% of the basal level is suggestive of renovascular hypertension and can be used as both a screening and a prognostic test regarding intervention. Oral captopril will also stimulate disproportionate renin production from the ischemic kidney and will therefore enhance the predictability of renal vein renin activity ratios.

Revascularization of the involved kidney with percutaneous transluminal angioplasty is recommended for younger patients with fibrous dysplasia of the renal artery. Only when percutaneous transluminal angioplasty is not technically feasible because of extensive disease in the branches of the renal artery is saphenous vein bypass grafting recommended. Sometimes complete surgical revascularization requires microvascular techniques that can only be performed ex vivo with autotransplantation of the kidney. The cure rate is 90% with proper selection, and the surgical mortality rate is < 1%. Medical treatment is always preferable to nephrectomy in young patients whose kidneys cannot be revascularized for technical reasons.

Compared with fibrous disease, atherosclerotic lesions respond less well to surgery and angioplasty, presumably because the patients are older and have more extensive vascular disease within the kidneys and throughout the vascular system. Hypertension may persist, and surgical complications are more common. Surgical mortality is higher than in young patients with fibrous dysplasia of the renal artery. Restenosis within 2 years of percutaneous transluminal angioplasty occurs in up to 50% of patients with atherosclerotic renal vascular disease, especially when the plaque is located at the

disposition to ventricular arrhythmias. Invasive electrophysiologic testing should be considered if noninvasive studies fail to identify suspected recurrent arrhythmic syncope. The role of electrophysiologic testing remains controversial except for unexplained recurrent syncope; a negative response defines a low-risk subgroup with a high rate of remission of syncope. Exercise testing is less valuable, unless physical activity precipitated the syncope. Tilt-table testing can identify vasodepressor or other reflex-induced syncope. Echocardiography may confirm suspected heart disease or prosthetic heart valve dysfunction. Image-intensification fluoroscopy is also valuable for the latter. Transesophageal echocardiography is valuable for diagnosing prosthetic heart valve dysfunction if transthoracic echocardiography is inclusive. Echocardiography can identify pericardial effusion and suggest cardiac tamponade.

Routine laboratory tests are of limited value, and additional studies should have specific goals. A fasting blood glucose can confirm hypoglycemia. An Hct can detect anemia, and hypokalemia or hypomagnesemia can be identified as a predisposing factor for arrhythmias. Serum troponin or creatinine phosphokinase elevation may identify the unusual case of acute MI presenting as syncope. The Po_2 is decreased, and there may be ECG evidence of acute cor pulmonale with pulmonary embolism; perfusion or ventilation scanning is excellent for screening. An EEG is warranted if a seizure disorder is suspected; CT and MRI of the head and brain are indicated for a focal neurologic deficit or if an intracranial process is suspected in the differential diagnosis.

syncopal episode should undergo more intensive evaluation for causes of syncope.

Prognosis and Treatment

In young persons without cardiovascular disease, syncope of unknown cause has a favorable prognosis, and elaborate evaluation is rarely required. In contrast, in the elderly, syncope may be due to the interaction of coexisting problems that may impair cardiovascular compensatory mechanisms. Typically, assuming the horizontal posture ends the syncopal episode, and no further immediate treatment is needed unless required by the underlying cause. Elevation of the legs more rapidly reestablishes cerebral perfusion. If the patient is allowed to sit up right too rapidly, syncope may recur; the problem is sometimes aggravated if the patient is propped upright or carried in the upright posture.

Bradyarrhythmias may require pacemaker implantation, and tachyarrhythmias require specific drug therapy. Implantable defibrillators may be needed for ventricular arrhythmias. Carotid sinus hypersensitivity may require pacemaker insertion for bradyarrhythmias, or carotid sinus radiation may alleviate the vasodepressor component. Management of volume depletion, hypoglycemia, anemia, electrolyte abnormalities, drug toxicity is standard. Elderly age does not contraindicate aortic valve surgery, the most common valvular surgery in the elderly. Hypertrophic cardiomyopathy with obstruction is treated with β-blockers, verapamil, or septal myomectomy; associated arrhythmias may respond to these treatments and to amiodarone.

201 / ARTERIOSCLEROSIS

A generic term for several diseases in which the arterial wall becomes thickened and loses elasticity.

Vascular disease, which affects the brain, heart, kidneys, other vital organs, and extremities, is the leading cause of morbidity and mortality in the USA and in most Western countries. There were almost 1 million deaths due to vascular disease in the USA in 1994 (twice as many as from cancer and times as many as from accidents). Although prevention and treatment of coronary artery disease (CAD) resulted in a 28.6% reduction in age-adjusted death rates between 1980 and 1994, CAD and ischemic stroke continue ing prevalence in the rest of the world.

The death rate from CAD among white men aged 25 to 34 is about 1/10,000; at age 55 to 64, it is nearly 1/100. This age relationship may be due to the time required for lesions to develop or to the duration of exposure to risk factors. The death rate from CAD among white men aged 35 to 44 is 6.1 times that among age-matched white women. For unknown reasons, the sex difference is less apparent in nonwhites.

Atherosclerosis is the most common and serious vascular disease. Nonatheromatous forms include arteriolosclerosis and Mönckeberg's arteriosclerosis.

ATHEROSCLEROSIS

A form of arteriosclerosis characterized by patchy subintimal thickening (atheromas) of medium and large arteries, which can reduce or obstruct blood flow.

The prevalence of clinical manifestations of atherosclerosis in general increases in postmenopausal women and begins to approach that in age-matched men.

Pathology and Pathogenesis

Atherosclerotic plaque consists of accumulated intracellular and extracellular lipid, smooth muscle cells, connective tissue, and glycosaminoglycans. The earliest detectable lesion of atherosclerosis is the fatty streak (consisting of lipid-laden foam cells, which are macrophages that have migrated from monocytes from the circulation into the subendothelial layer of the intima), which later evolves into the fibrous plaque (consisting of intimal smooth muscle cells surrounded by connective tissue and intracellular and extracellular lipids).

Atherosclerotic vessels have reduced systolic expansion and abnormally rapid wave propagation. Arteriosclerotic arteries of hypertensive persons also have reduced elasticity, which is further reduced when atherosclerosis develops.

Two main hypotheses have been proposed to explain the pathogenesis of atherosclerosis: the lipid hypothesis and the chronic endothelial injury hypothesis. They are probably interrelated.

etration of LDL into the arterial wall, leading to lipid accumulation in smooth muscle cells and in macrophages (foam cells). LDL also augments smooth muscle cell hyperplasia and migration into the subintimal and intimal region in response to growth factors. LDL is modified or oxidized in this environment and is rendered more atherogenic. Small dense LDL cholesterol particles are also more susceptible to modification and oxidation. The modified or oxidized LDL is chemotactic to monocytes, promoting their migration into the intima, their early appearance in the fatty streak, and their transformation and retention in the subintimal compartment as macrophages. Scavenger receptors on the surface of macrophages facilitate the entry of oxidized LDL into these cells, transferring them into lipid-laden macrophages and foam cells. Oxidized LDL is also cytotoxic to endothelial cells and may be responsible for their dysfunction or loss from the more advanced lesion.

An atherosclerosis model has been studied in monkeys fed a cholesterol-rich diet. Within 1 to 2 wk of inducing hypercholesterolemia, monocytes become attached to the surface of the arterial endothelium through the induction of specific receptors, migrate into the subendothelium, and accumulate lipid (hence, foam cells). Proliferating smooth muscle cells also accumulate lipid. As the fatty streak and fibrous plaque enlarge and bulge into the lumen, the subendothelium becomes exposed to the blood at sites of endothelial retraction or tear, and platelet aggregates and mural thrombi form. Release of growth factors from the aggregated platelets may increase smooth muscle proliferation in the intima. Alternatively, organization and incorporation of the thrombus into the atherosclerotic plaque may contribute to its growth.

The chronic endothelial injury hypothesis postulates that endothelial injury by various mechanisms produces loss of endothelium, adhesion of platelets to subendothelium, aggregation of platelets, chemotaxis of monocytes and T-cell lymphocytes, and release of platelet-derived and monocyte-derived growth factors that induce migration of smooth muscle cells from the media into the intima, where they replicate, synthesize connective tissue and proteogly-

cans, and form a fibrous plaque. Other cells (eg, macrophages, endothelial cells, arterial smooth muscle cells) also produce growth factors that can contribute to smooth muscle hyperplasia and extracellular matrix production.

These two hypotheses are closely linked and not mutually exclusive. Modified LDL is cytotoxic to cultured endothelial cells and may induce endothelial injury, attract monocytes and macrophages, and stimulate smooth muscle growth. Modified LDL also inhibits macrophage mobility, so that once macrophages transform into foam cells in the subendothelial space they may become trapped. In addition, regenerating endothelial cells (after injury) are functionally impaired and increase the uptake of LDL from plasma.

The atherosclerotic plaque may grow slowly and over several decades may produce a severe stenosis or may progress to total arterial occlusion. With time, the plaque becomes calcified. Some plaques are stable, but others, especially those rich in lipids and inflammatory cells (eg, macrophages) and covered by a thin fibrous cap, may undergo spontaneous fissure or rupture, exposing the plaque contents to flowing blood. These plaques are deemed to be unstable or vulnerable and are more closely associated to the onset of an acute ischemic event. The ruptured plaque stimulates thrombosis; the thrombi may embolize, rapidly occlude the lumen to precipitate a heart attack or an acute ischemic syndrome, or gradually become incorporated into the plaque, contributing to its stepwise growth.

Risk Factors

Major nonreversible risk factors for atherosclerosis include age, male sex, and family history of premature atherosclerosis. Major reversible risk factors are discussed below. Evidence also strongly suggests that physical inactivity is associated with an increased risk of CAD. Although personality type has been proposed as a risk factor, its role is controversial.

Abnormal serum lipid levels: Elevated levels of low density lipoprotein (LDL) and reduced levels of high density lipoprotein (HDL) predispose to atherosclerosis. The association of total serum cholesterol and LDL cholesterol levels with the risk of CAD is direct and continuous. HDL levels are inversely correlated with CAD risk. The main causes of reduced HDL are cigarette smoking, obesity, and physical inactivity. Low HDL is also associated with the use of androgenic and related steroids (including anabolic steroids), β-blockers, hypertriglyceridemia, and genetic factors.

Cholesterol level and CAD prevalence are influenced by genetic and environmental factors (including diet). Persons with low serum cholesterol levels who move from a country with a low CAD prevalence to a country with a high CAD prevalence and who tend to alter their eating habits accordingly develop higher serum cholesterol levels and an increased risk of CAD.

Hypertension: High diastolic or systolic BP is a risk factor for stroke, MI, and cardiac and renal failure. The risk associated with hypertension is lower in societies with low average cholesterol levels.

Cigarette smoking: Smoking increases the risk of peripheral artery disease, CAD, cerebrovascular disease, and graft occlusion after reconstructive arterial surgery. Smoking is particularly hazardous in persons at increased cardiovascular risk. There is a dose relationship between the risk of CAD and the number of cigarettes smoked daily. Passive smoking may also increase the risk of CAD. Men and women are both susceptible, but the risk for women may be greater. Nicotine and other tobacco-derived chemicals are toxic to vascular endothelium.

Cigarette smoking increases LDL and decreases HDL levels, raises blood carbon monoxide (and could thereby produce endothelial hypoxia), and promotes vasoconstriction of arteries already narrowed by atherosclerosis. It also increases platelet reactivity, which may favor platelet thrombus formation, and increases plasma fibrinogen concentration and Hct, resulting in increased blood viscosity.

Diabetes mellitus: Both insulin-dependent and non–insulin-dependent diabetes mellitus are associated with earlier and more extensive development of atherosclerosis as part of widespread metabolic derangement that includes dyslipidemia and glycosylation of connective tissue. Hyperinsulinemia damages vascular endothelium. Diabetes is a particularly strong risk factor in women and significantly negates the protective effect of female hormones.

Obesity: Some studies have found that obesity, particularly truncal obesity in men, is an independent risk factor for CAD. Hypertriglyceridemia is commonly associated with obesity, diabetes mellitus, and insulin resistance and appears to be an important independent risk factor in persons with lower LDL or HDL levels and in the nonelderly. Not all triglyceride elevations are likely to be atherogenic. Smaller, denser very low density lipoprotein particles may carry greater risk.

Physical inactivity: Several studies have associated a sedentary lifestyle with increased CAD risk, and others have shown that regular exercise may be protective.

Hyperhomocysteinemia: Elevated blood homocysteine due to a genetically determined decrease in its metabolism may cause vascular endothelial injury, which predisposes the vessels to atherosclerosis (see also Ch. 202 and Hyperhomocysteinemia in Ch. 132).

***Chlamydia pneumoniae* infection:** *Chlamydia pneumoniae* infection or viral infection may play a role in endothelial damage and chronic vascular inflammation that may lead to atherosclerosis.

Symptoms and Signs

Atherosclerosis is characteristically silent until critical stenosis, thrombosis, aneurysm, or embolus supervenes. Initially, symptoms and signs reflect an inability of blood flow to the affected tissue to increase with demand (eg, angina on exertion, intermittent claudication). Symptoms and signs commonly develop gradually as the atheroma slowly encroaches on the vessel lumen. However, when a major artery is acutely occluded, the symptoms and signs may be dramatic. Specific ischemic disorders related to occlusion are described elsewhere in §16 and in Ch. 174.

Diagnosis

Atherosclerosis is suspected based on the risk factors and on its symptoms and signs, for which there may be few. Atheromatous obstruction is commonly confirmed by arteriography or Doppler ultrasonography. Diagnosis of specific manifestations (eg, CAD) is described elsewhere in THE MANUAL.

Hyperlipidemia (see also Ch. 15) commonly presents with symptoms and signs of premature obliterative atherosclerosis affecting the brain (cerebral transient ischemic attacks or stroke), heart (angina pectoris or MI), intestine, and lower extremities (intermittent claudication). Xanthomas (in the creases of hands and elbows and along tendon sheaths) and xanthelasmas are sometimes associated with hyperlipidemia, particularly of the familial type. Recurrent attacks of acute pancreatitis, with or without alcoholism, suggest hypertriglyceridemia. A family history of hyperlipidemia or onset of cardiovascular disease before age 60 is further reason to look for premature atherosclerosis.

Prevention

The most effective way to prevent the cardiovascular and cerebrovascular complications of atherosclerosis and the associated arterial thrombosis is to prevent atherosclerosis itself. Reversible risk factors for atherosclerosis are abnormal serum lipid levels, hypertension, cigarette smoking, diabetes mellitus, obesity, physical inactivity, hyperhomocysteinemia, and possibly *C. pneumoniae* infection. Increased understanding of these risk factors and their role in the etiology, pathogenesis, and course of atherosclerosis will lead to more focused intervention for preclinical or overt atherosclerotic disease and will thereby contribute to further declines in morbidity and mortality.

Abnormal serum lipid levels: At least 20 randomized trials show that lowering serum LDL cholesterol levels slows progression or induces regression of CAD and reduces coronary events. The benefits are greatest in patients at greater risk of CAD (ie, those with other risk factors, eg, hypertension, cigarette smoking) and in those with the most elevated cholesterol levels. Lowering serum LDL is also beneficial in those with preexisting CAD, even if their LDL levels are not elevated. Recent trials have shown a significant decrease in cardiovascular and total mortality when the statins are used to lower cholesterol. Statins also slow the progression of CAD (shown by angiography) in patients with arterial bypass grafts and elevated LDL cholesterol levels. Guidelines for screening and treatment of mild, moderate, and severe hypercholesterolemia are discussed in Ch. 15.

Hypertension: Treatment of patients with elevated BP reduces stroke and overall mortality, but the effect on coronary event reduction is less striking. Pooled analysis of all studies of BP lowering shows a risk reduction of 40% in stroke, 8% in MI, and 10% in cardiovascular mortality.

Cigarette smoking: Smoking cessation should be encouraged whenever possible. The risk in persons who quit, regardless of how long they smoked, is half of that in those who continue to smoke. Smoking cessation also decreases morbidity and mortality in patients with peripheral vascular disease and decreases mortality after coronary bypass surgery and in post-MI patients.

Diabetes mellitus: Although tight glycemic control reduces the risk of microvascular complications of diabetes, the effects on macrovascular disease and atherosclerosis are less clear. Hyperlipidemia and hypertension are more common in diabetics, and these risk factors together with hyperinsulinemia may contribute to the increased CAD risk.

Obesity: Weight loss raises HDL levels and should be encouraged when possible.

Physical inactivity: Several randomized trials have demonstrated that moderate exercise performed consistently reduces the clinical manifestations and mortality of CAD in high-risk patients. Regular exercise has also been reported to lower the incidence of MI and death, but it is uncertain whether the association is causal or merely indicates that healthier persons are more likely to exercise regularly. Regular exercise increases HDL levels and can lower BP.

Hyperhomocysteinemia: Hyperhomocysteinemia in the presence or absence of low plasma concentrations of vitamin B can be corrected by folate administration with or without vitamin B supplementation. However, it is unclear whether this treatment is beneficial.

***Chlamydia pneumoniae* infection:** Understanding of the role of infection and inflammation in atherosclerosis and its complications is improving. Trials are underway to assess whether antibiotic treatment will impact the infection's clinical manifestations.

Treatment

Treatment of established atherosclerosis is directed at its complications (eg, angina pectoris, MI, arrhythmias, heart failure, kidney failure, ischemic stroke, and peripheral arterial occlusion). These subjects are covered elsewhere in THE MANUAL.

NONATHEROMATOUS ARTERIOSCLEROSIS

In arteriosclerosis of the aorta and its major branches, fibrosis and some intimal thickening develop with aging, with weakening and disruption of the elastic lamellae. The media (smooth muscle coat) atrophies to a certain extent, and the lumen of the aorta or one or more of its branches widens (ectasia), possibly leading to aneurysm. Hypertension plays a major role in aortic atherosclerosis and aneurysm formation. Possible intimal injury, ectasia, and ulceration may lead to thrombus formation, embolism, or complete vessel occlusion.

Overall loss of elasticity in the vessel wall may weaken the vessel and predispose to longitudinal dissection of blood along the laminar planes of the vessel in a process called aortic dissection (see Ch. 211).

Arteriolosclerosis describes hypertrophy of the media and subintimal fibrosis with hyaline degeneration that develops in small muscular arteries or arterioles. Hypertension is a major factor.

In **Mönckeberg's arteriosclerosis** (medial calcific sclerosis), spotty degeneration occurs in later years in the smooth muscle of the media, with focal calcification and even bone formation. Sometimes the vessel is converted for some length into a rigid calcified tube, without narrowing the lumen and is of little clinical consequence.

202 / CORONARY ARTERY DISEASE

Most coronary artery disease (CAD) is due to subintimal deposition of atheromas in the large and medium-sized arteries serving the heart. Risk factors and the pathogenesis of atherosclerotic lesions and CAD are discussed in Chs. 15 and 201. Less often, CAD is due to coronary spasm, which is usually idiopathic (with or without associated atheroma) or may be due to drugs such as cocaine. Rare causes include an embolus to the coronary artery, Kawasaki syndrome (see Ch. 265), and vasculitis (eg, in SLE).

Coronary atherosclerosis is characteristically insidious in onset, is often irregularly distributed in different vessels, and can abruptly interfere with blood flow to segments of the myocardium, most often due to rupture of an eccentric atheromatous plaque with consequent intraluminal thrombosis.

The major complications of CAD are angina pectoris, unstable angina, MI, and sudden cardiac death due to arrhythmias. In the USA, CAD is the leading cause of death in both sexes, accounting for about one third of deaths each year.

Although the precise pathogenesis of CAD is unclear, the risk factors are well known: high blood levels of low density lipoprotein cholesterol (LDL-C) and lipoprotein a, low blood levels of high density lipoprotein cholesterol (HDL-C) and serum vitamin E, and poor physical fitness. High blood levels of triglycerides and insulin reflecting insulin resistance may be risk factors, but the data are less clear. CAD risk is increased by tobacco use; diets high in fat and calories and low in phytochemicals (found in fruits and vegetables), fiber, and vitamin E and C or, at least in some persons, diets with relatively low levels of omega-3 polyunsaturated fatty acids (PUFAs); poor stress management; and inactivity. Several systemic diseases (eg, hypertension, diabetes, hypothyroidism) are also associated with increased CAD risk.

Recent studies have shown an association between CAD and a common variant of the platelet fibrinogen receptor (PI^{A2}), found in 20% of Americans. The presence of this variant may be as strong a predictor of CAD as cigarette smoking and hypertension. Whether giving antiplatelet therapy to persons with this variant can prevent CAD remains to be established.

Homocysteine has recently been identified as a risk factor for coronary, peripheral, and cerebral vascular disease. Patients with homocystinuria, a rare recessive disease, have plasma homocysteine levels 10 to 20 times above normal (hyperhomocysteinemia) and accelerated, premature vascular disease. Homocysteine has a direct toxic effect on endothelium and promotes thrombosis and oxidation of LDL. Normal values range from about 4 to 17 μmol/L. Modest elevations of total plasma homocysteine have multiple causes, including low levels of folic acid, vitamins B_6 and B_{12}, renal insufficiency, certain drugs, and genetically controlled variations in homocysteine metabolic enzymes. Patients with homocysteine values in the top 5% have a 3.4 greater risk of MI or cardiac death than those in the lower 90% after adjustment for other risk factors. Increased homocysteine levels are associated with increased risk regardless of etiology. Recent studies suggest a graded risk even in normal-range homocysteine; thus, reduction of normal plasma levels may be advantageous. The most simple and effective way to reduce plasma homocysteine is administration of folic acid 1 to 2 mg/day, which has essentially no side effects except in untreated vitamin B_{12} deficiency. Many authorities recommend that patients with CAD be screened for plasma homocysteine levels and, unless the values are in the lower normal range, treatment be initiated with folic acid. (See also Hyperhomocysteinemia in Ch. 132.)

Patients with CAD undergoing atherectomy have biologic markers suggesting coronary artery localization of *Chlamydia* infection. The role of this and other putative infectious agents in the genesis of CAD is being investigated.

PREVENTION OF CORONARY ARTERY DISEASE

CAD prevention usually begins with reversal of modifiable risk factors. Smoking cessation is of primary importance. Additional strategies include dietary modification, achievement of appropriate weight for height, proper management of stress, and regular exercise. Physicians should treat coexisting disorders associated with increased risk, such as hypertension (see Ch. 199), hypercholesterolemia, diabetes (see Ch. 13), or hypothyroidism (see Ch. 8). In particular, aggressive cholesterol lowering with HMG-CoA reductase inhibitors (statins—see also Ch. 15) has now been demonstrated to save lives, prevent unstable angina and MI, and decrease coronary revascularization rates.

DIETARY MODIFICATION

Fats: The average U.S. diet contains 37% of total calories as fat. The American Heart

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REVIEW ARTICLE

Hypertension and the prothrombotic state

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The basic underlying pathophysiological processes underlying the major complications of hypertension (that is, heart attacks and strokes) are thrombogenesis and atherogenesis. Indeed, despite the blood vessels being exposed to high pressures in hypertension, the complications of hypertension are paradoxically thrombotic in nature rather than haemorrhagic. The evidence suggests that hypertension appears to confer a prothrombotic or hypercoagulable state, which can be related to conventional risk factors, target organ damage, complications and long-term prognosis, as well as different antihypertensive treatments. Further work is needed to examine the mechanisms leading to this phenomenon, the potential prognostic and treatment implications, and the possible value of measuring these parameters in routine clinical practice.
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Keywords: hypercoagulable; prothrombotic; coagulation; haemorheology; prognosis

Introduction

Hypertension is well-recognised to be an important contributor to heart attacks and stroke.[1] Furthermore, effective antihypertensive therapy reduces strokes by 30–40%, and coronary artery disease by approximately 25%.[2] Nevertheless the basic underlying pathophysiological processes underlying both of these major complications of hypertension are thrombogenesis and atherogenesis. Indeed, despite the blood vessels being exposed to high pressures in hypertension, the complications of hypertension are paradoxically thrombotic in nature rather than haemorrhagic. Whilst much attention has been focused on the renin-angiotensin system, cathecholamines and other neurohormonal mechanisms involved in the pathogenesis of hypertension, the study of the prothrombotic state in hypertension has been relatively neglected.

Over 150 years ago, Virchow postulated that three features predispose to thrombus formation, that is, abnormalities in blood flow, blood constituents and the vessel wall.[3] Whilst Virchow was referring to venous thrombosis, the concepts can be applied to arterial thrombosis. An update of Virchow's triad for thrombogenesis for the new millennium can be considered by reference to abnormalities of haemorheology and turbulence at bifurcations and stenotic regions (that is, 'abnormal blood flow'), abnormalities in platelets as well as the coagulation and fibrinolytic pathways ('abnormal blood constituents') and finally, abnormalities in the endothelium ('abnormal vessel wall').[4]

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Indeed, patients with hypertension are well-recognised to demonstrate abnormalities of each of these components of Virchow's triad, leading to a prothrombotic or hypercoagulable state.[4] Furthermore, the processes of thrombogenesis and atherogenesis are intimately related, and many of the basic concepts thrombogenesis can be applied to atherogenesis. Importantly, recent improvements in biochemical techniques have enabled us to quantify different components of both these processes.

Evidence for the prothrombotic state in hypertension

Evidence for the hypercoagulable state in hypertension has been extensively reviewed.[4–6] Indeed, evidence from numerous epidemiological,[7–9] cross-sectional[10,11] and cohort studies[12,13] have reported abnormalities in the coagulation and fibrinolytic pathways, as well as in platelets and the endothelium.

Relation to conventional risk factors

These abnormalities in haemostasis appear to be additive to conventional risk factors for cardiovascular and cerebrovascular events. For example, in the ECAT study, high plasma fibrinogen in association with high serum cholesterol was associated with the highest risk for cardiovascular events.[14] The interaction between plasma fibrinogen and cholesterol levels is also demonstrated in the Leigh Study, where the incidence of heart attacks was six times greater in those with high plasma fibrinogen ($\geq$3.5 g/l) and cholesterol levels ($\geq$6.2 mmol/l), when compared to those with low fibrinogen ($<$3.5 g/l) levels.[15] Other risk factors, such as smoking and diabetes, markedly influence the prothrombotic state and are probably additive to the intrinsic

abnormalities (and cardiovascular risk) seen in hypertensives.

Association with target organ damage

The abnormalities in haemostasis in hypertensives can be related to target organ damage, such as the presence of left ventricular hypertrophy on echocardiography.[10] The latter is a powerful predictor of cardiovascular events, with an eight-fold increase in the risk of stroke and a four-fold increase in the risk of coronary artery disease. The presence of left ventricular hypertrophy (LVH) is also an independent contributor to the risk of stroke in atrial fibrillation.[16] Furthermore, high von Willebrand factor levels, an established index of endothelial damage or dysfunction can be related to microalbuminuria (defined as the excretion of urine albumin between 20 and 200 mcg/min), another surrogate manifestation of hypertensive target organ damage.[17]

The abnormalities in various indices can perhaps be related to the degree, and possibly the duration of hypertension, and those with mild hypertension or lower blood pressures, and more recent onset hypertension (which is usually more difficult to precisely quantify) may show less abnormalities in the prothrombotic state. For example, patients with severe hypertension (defined as >160/95 mm Hg) demonstrate high plasma von Willebrand factor levels[10,18] which does not appear to be present in patients with milder elevations of blood pressure.[7,8] Although endothelial dysfunction or damage can be present as a result of hypertension, others have even considered that endothelial damage may actually promote hypertension.[19]

Association with the complications of hypertension

The common complications of hypertension can also be related to a prothrombotic state. For example, hypertension is a common cause of atrial fibrillation[20] and indeed, is additive to the risk of stroke and thromboembolism with this arrhythmia.[21] Atrial fibrillation *per se* is also well-recognised to be associated with abnormalities of haemostasis and endothelial dysfunction, which are altered by cardioversion and antithrombotic therapy, and are independent of underlying aetiology or structural heart disease.[22]

Hypertension is an important cause of heart failure[23] and the evidence also points towards a hypercoagulable state in heart failure.[24] Indeed, heart failure is an important contributor to stroke and thromboembolism, with an inverse relationship between ejection fraction and stroke in the Survival and Ventricular Enlargement (SAVE) study; there was an 18% increase in stroke risk for every 5% reduction in left ventricular ejection fraction, thus clearly relating thromboembolism to severe cardiac impairment and the severity of heart failure.[25]

Association with prognosis

Mounting evidence of the prognostic value of these markers raises the possibility that they are not merely markers or consequences of atherothrombotic disease, but may contribute to the pathogenesis of hypertension and its complications. Indeed various indices are predictive of outcome in hypertension.

For example the Leigh general practice study reported that hypertensive subjects with plasma fibrinogen levels >3.5 g/l had a 12-fold higher cardiovascular risk than those with plasma fibrinogen levels <2.9 g/l.[15] Blann *et al*[26] suggested that high von Willebrand factor levels had prognostic value in hypertension, being predictive of cardiovascular disease progression. The study by Agewall *et al*[12] found that prothrombin fragment 1+2 and C-reactive protein were independent predictors of major coronary events. Our recent study also suggested that patients with hypertension who developed cardiovascular or cerebrovascular events at 4 years' follow-up had higher baseline vWf and fibrin D-dimer levels compared to those without events, although on Cox multivariate proportional hazards analysis only plasma fibrinogen and blood pressure levels emerged as independent predictors.[13]

The possibility therefore remains that some prothrombotic indices, either individually or combination, may provide sufficiently high predictive value for cardiovascular disease and stroke. Further prospective studies on large cohorts would be required to confirm this hypothesis.

Effects of treatment

Treating hypertension may reduce the prothrombotic state. Indeed, antihypertensive agents with particular benefits in the hypercoagulable state in hypertensives would be likely to have additional advantages in reducing stroke and other thromboembolic events. For example, treated hypertensives demonstrate normal von Willebrand factor levels.[18] However, different drugs may affect the prothrombotic state differently (as reviewed by Lee[6]). For example, drugs such as beta-blockers or calcium antagonists may have favourable haemorheological actions. In contrast, diuretics may have the opposite effect in increasing blood viscosity.[27,28] These differences may in part explain some of the differences between different antihypertensive agents in the reduction of endpoints in some trials of antihypertensive therapy. For example, thiazides are beneficial in older hypertensives (over beta-blockers and placebo) in reducing stroke and cardiac events.[29,30] In contrast, hypertensive patients on diuretic therapy have an increased mortality if electrocardiographic abnormalities (including LVH) are present.[31]

Another example is isolated systolic hypertension, which was regarded as a 'different' disease from conventional systolic-diastolic hypertension. Indeed, most epidemiological and treatment studies have concentrated on diastolic blood pressures, whilst it has been recognised that systolic blood pressure is a better predictor of cardiovascular events.[32,33] Furthermore, recent trials have confirmed the value of treating isolated systolic hypertension.[34,35] Indeed data from the Syst-Eur study[35]

demonstrates how devastating isolated systolic hypertension can be, in terms of the number of thrombosis-related complications (that is, strokes and heart attacks) in the placebo group, which was reduced by antihypertensive therapy. This has led to recent appeals for the abandonment of the measurement of diastolic blood pressure.[36] Both isolated systolic hypertension and systolic-diastolic hypertension have been shown to be associated with abnormalities of haemorheology, thrombogenesis and endothelial dysfunction, as well as having similar echocardiographic parameters and left ventricular mass index, in keeping with both disease processes being similar in pathophysiology.[11]

Cause or effect?

It is likely that a continuum exists between normality, 'statistically increased' levels of haemostatic markers and overt thrombosis. If so, it is also likely that those with high levels of haemostatic markers are predictive of subsequent thromboembolic events, which has been borne out by recent published evidence. Indeed, other haemostatic markers have been shown to have prognostic implications in patients with ischaemic heart disease and peripheral arterial disease.[37]

Nevertheless this raises the question whether the abnormal prothrombotic indices are 'cause or effect' with regard to cardiovascular disease. Whilst elevated plasma levels of a prothrombotic state are consistently associated with various cardiovascular disorders (coronary, cerebrovascular and peripheral artery disease) and the risk of vascular events, it has been suggested that these associations may be explained by a reactive or secondary rise in these plasma haemostatic factors, either as an acute phase response or as an atherosclerosis-related 'haematological stress syndrome'.[38] Since the processes of thrombogenesis and atherogenesis have certain similarities to inflammatory disease, the elevations in various indices may reflect the severity of vascular disorders as a secondary phenomenon rather than act as a true prognostic factor.

The hereditary determination of levels of some clotting markers makes it less likely that raised levels are simply a secondary response to cardiovascular disorders. For example, raised plasma levels of some indices, such as fibrinogen and vWf are also known to precede cardiovascular events. In addition, there is also an association between plasma fibrinogen or vWf levels with the clinical severity of angina or degree of coronary artery disease.[39] High levels of markers such as vWf are found following endothelial injury by smoking, hypertension or hyperlipidaemia.[40] There is also experimental evidence that some prothrombotic indices may be increased by glucocorticoids and cytokines such as interleukin-1 and tumour necrosis factor (TNF) which are produced by monocytes and macrophages.[41,42] However, since some clotting factors are also acute phase proteins, increased levels may simply reflect endothelial activation or stimulation, and not endothelial dysfunction. The precise mechanisms for the elevated levels of various prothrombotic markers in hypertension and other cardiovascular disorders therefore remain uncertain, although a cytokine-mediated increase in synthesis is likely to be the common pathway.

Conclusion

Whilst the blood vessels are exposed to high pressures in patients with hypertension, the main complications related to hypertension (that is, heart attacks and stroke) are paradoxically thrombotic in nature. Hypertension appears to confer a prothrombotic or hypercoagulable state, which can be related to target organ damage, long-term prognosis and treatment. Further work is needed to examine the mechanisms leading to this phenomenon, the potential prognostic and treatment implications, and the possible value of measuring these parameters in routine clinical practice. This new millennium may provide the answers.

Acknowledgements

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Evidence of platelet activation in hypertension

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To test the hypothesis that platelet activation is present in hypertension, we measured plasma markers beta thromboglobulin and soluble P-selectin in hypertensive patients and normotensive controls. Both markers were raised in the patients ($P < 0.05$), and in a subgroup of patients, beta thromboglobulin was reduced with successful treatment of hypertension with the ACE inhibitor quinapril. We suggest that reversible platelet activation is present in hypertension. This may be a contributing factor to the link between this risk factor and the development of thrombotic disease such as stroke.

Keywords: soluble P-selectin; beta thromboglobulin; platelets

Introduction

A major consequence of hypertension is stroke. However, it is curious that these strokes are often of thrombotic/occlusive origin, and not haemorrhagic origin. It has therefore been hypothesised that this may be related to changes in thrombosis and haemostasis (eg, levels of fibrinogen, cross-linked fibrin D-dimer) in patients with hypertension. An additional manifestation of these changes which could promote thrombosis may also be inappropriate changes in platelet physiology such as excess activation and increased volume, as is known in ischaemic heart disease and stroke.[1] Increased plasma levels of platelet specific products soluble P-selectin (a component of the alpha granule membrane) and beta thromboglobulin (a constituent of the alpha granule matrix) are taken to imply increased platelet activation.[2,3] We therefore aimed to determine whether or not patients with hypertension would have evidence of platelet activation as defined by increases in these soluble plasma markers.

Subjects and methods

We measured soluble P-selectin in citrated plasma and beta thromboglobulin in CTAD plasma (a cocktail of citrate, theophylline, adenosine and dipyridamole designed to minimise *ex vivo* platelet activation: Diatube, Diagnotica Stago, France) by commercial immunoassay in two separate studies. The first was a cross-sectional study of 100 patients with essential hypertension (mean systolic blood pressure [SBP] 162 mm Hg, mean diastolic blood pressure [DBP] 98 mm Hg, 76 men, mean age 54 years) and 47 normotensive (blood pressures 138/77 mm Hg, 30 men, mean age 52 years) age and sex matched controls. Soluble P-selectin was measured by the Takara Shuzo ELISA (Honshu, Japan). The second was a study of 40 patients with newly-diagnosed hypertension (mean SBP 165 mm Hg, mean DBP 107 mm Hg, 32 men, mean age 48 years). Beta thromboglobulin was measured by Amersham RIA (Amersham, UK) and this study was controlled by plasma from 22 normotensive (mean blood pressure 135/74 mm Hg), age (45 years) and sex (15 men) matched controls. Twenty of the patients were treated with the angiotensin-converting enzyme (ACE) inhibitor quinapril 10 mg daily. A second plasma sample was obtained from the same patients after 12 weeks, when BP had fallen significantly to a mean of systolic 151 mm Hg ($P < 0.05$) and diastolic 98 mm Hg ($P < 0.05$). All subjects were asymptomatic for vascular disease and were also free of complications such as diabetes, renal and liver disease, or connective tissue disease.

Statistics

Data between patients and controls was analysed by the Mann-Whitney U test. Data at two time points was analysed by paired *t*-testing. Correlations were by Spearman's ranks method.

Results

In the cross-section study, soluble P-selectin in the citrated plasma of the patients was median 300 ng/mL, range 190–800 ng/mL. In the controls it was median 228, range 175–412 ng/mL ($P < 0.05$, Figure 1). However, there were no Spearman rank correlations with either SBP or DBP.

In the second study plasma beta thromboglobulin was median 58 ng/ml (95% confidence interval [CI] 40–77 ng/mL) in the patients and 32 ng/mL (95% CI 27–36 ng/mL) in 22 controls ($P < 0.01$, Figure 2). Again, there were no Spearman rank correlations with either SBP or DBP.

After 12 weeks treatment with quinapril, mean BP in the 20 patients dropped to 151/98 ($P < 0.05$). This was accompanied by a reduction in plasma beta

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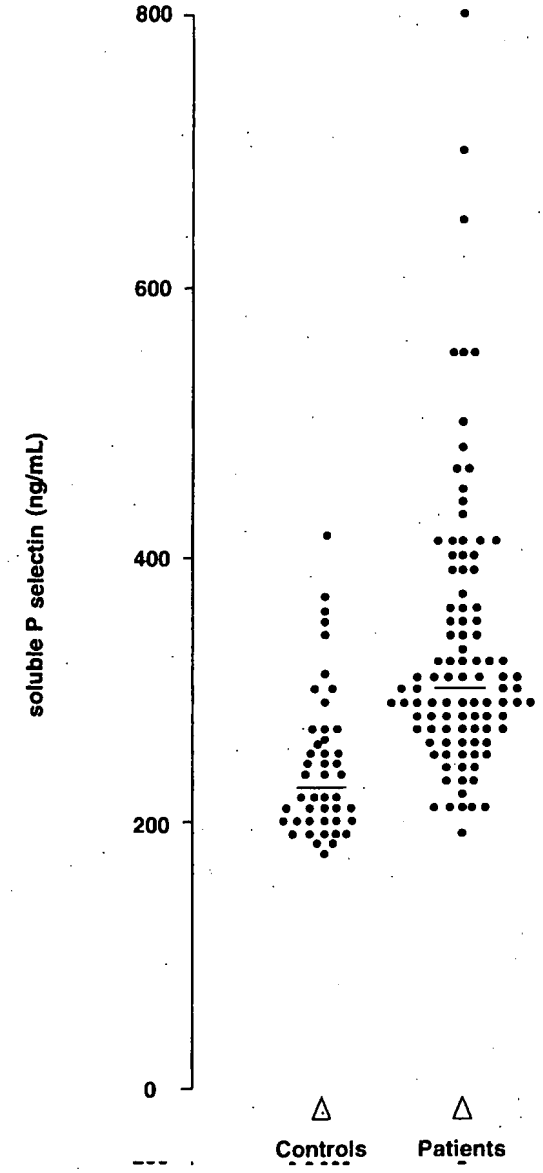

Figure 1 Levels of soluble P-selectin in the plasma of patients with hypertension and in controls. The bar is the median value.

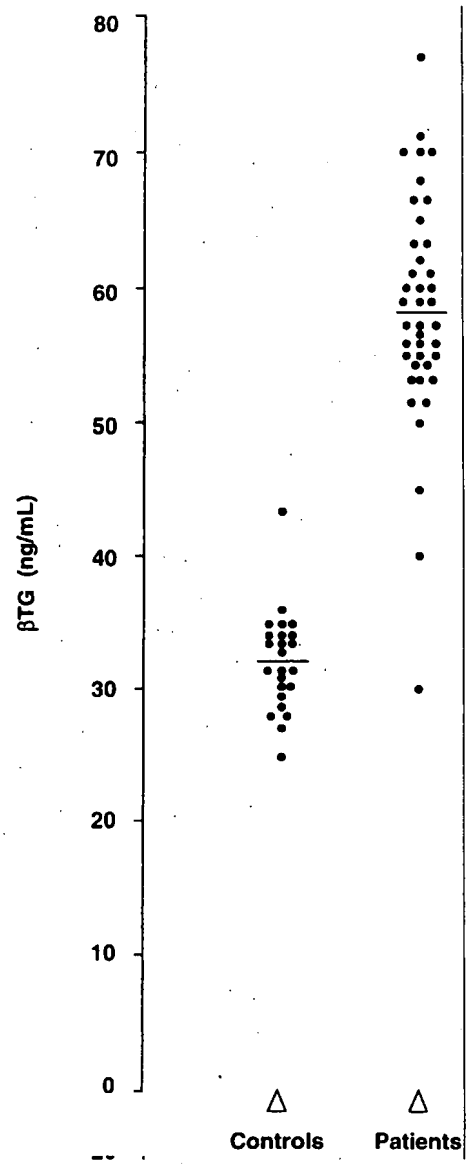

Figure 2 Levels of beta thromboglobulin (βTG) in the plasma of patients with hypertension and in controls. The bar is the median value.

thromboglobulin from a median of 69 ng/mL (95% CI 45–106 ng/mL) to a median of 46 ng/mL (95% CI 35–60 ng/mL) ($P < 0.05$).

Discussion

The majority of strokes due to hypertension are thrombotic, implicating changes in thrombosis, haemostasis and/or platelet function. Beta thromboglobulin and soluble P-selectin are plasma markers of platelet activation.[2,3] Therefore, the raised levels in essential hypertension we have found suggests adverse changes to the physiology of this cell. In addition, our data point to evidence of reversible platelet activation in patients with hypertension. Our data compliment previous studies of the effects of captopril on platelet aggregation.[4,5] Both these studies showed that the drug produced a beneficial profile in the response of platelets to *ex vivo* aggregation by adrenaline, ADP and collagen. We cannot say if this effect is likely to be constant for any particular therapy as we and others have used only ACE inhibitors.

Together, changes may be at least partly responsible for the increased risk of thrombotic stroke and indicates that therapeutic strategies aimed at reducing platelet activity (such as the use of aspirin) may be beneficial.

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Polymorphonuclear Leukocyte-Platelet Interaction: Role of P-Selectin in Thromboxane B_2 and Leukotriene C_4 Cooperative Synthesis

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Summary

In PMN/platelet suspensions stimulated by fMLP giant mixed aggregates are formed and TxB_2 and LTC_4 are synthesized as the result of the cooperation in the arachidonic acid (AA) metabolism during cell/cell contact. PMN-derived cathepsin G induced the expression of P-selectin on platelet surface. GE12, an antibody against P-selectin, significantly reduced mixed cell aggregates. GE12 did not affect platelet aggregation induced by PMN-derived supernatants, indicating that the inhibitory effect of GE12 on mixed cell aggregation depends on inhibition of PMN/platelet adhesion. GE12 significantly reduced TxB_2 and LTC_4 production in PMN/platelet mixed cell suspensions stimulated by fMLP. As previously reported, synthesis of ^{3}H-TxB_2 in ^{3}H-AA-labeled PMN/unlabeled platelets indicates that platelets utilize ^{3}H-AA from PMN. ^{3}H-LTC_4 production in unlabeled PMN/^{3}H-AA-labeled platelets indicates that bidirectional routes are utilized in this system for LTC_4 synthesis. GE12 significantly reduced ^{3}H-TxB_2 and ^{3}H-LTC_4 synthesis. These results show that cathepsin G released by activated PMN induces the expression of P-selectin on platelet membrane: this adhesive glycoprotein modulates cell-cell contact and transcellular metabolism of AA.

Introduction

Polymorphonuclear leukocytes (PMN) and platelets cooperate in processing arachidonic acid (AA) or AA-derived intermediate metabolites into biologically active substances that play a pathophysiological role in inflammation and thrombosis (1, 2).

Human PMN activated in vitro by several specific agonists are able to activate coincubated autologous platelets. This effect is largely mediated by cathepsin G, a neutral serine protease released from azurophilic granules of activated PMN (3–8). In this system after challenge with n-formyl-methionyl-leucyl-phenylalanine (fMLP), activated cells form giant mixed aggregates composed of both cell types tightly interacting at membrane level as shown by electron microscopy (7).

In a previous study, we demonstrated that in experimental conditions, in which fMLP-challenged PMN were able to stimulate coincubated platelets through released cathepsin G, transcellular metabolism occurred in which platelets used PMN-derived unmetabolized AA to synthesize thromboxane (Tx) B_2 (9). In these experiments direct platelet/PMN contact was important for transcellular TxB_2 production.

Further investigations showed that in this system leukotriene (LT) C_4 is also formed. This metabolite may be generated by platelets (10) utilizing LTA_4 derived from activated PMN. Moreover part of this LTA_4 is result of the PMN metabolization of platelet-derived arachidonic acid, as shown by the appearance of ^{3}H-LTC_4 from PMN-^{3}H-AA-labeled platelets mixed cell suspensions activated by fMLP. This bidirectional pathway has been previously documented (11, 12).

Palmantier and Borgeat (13) rose the hypothesis that direct cell-cell contact by specific adhesion molecules may facilitate AA transcellular metabolism between platelets and PMN.

Prevention of PMN-endothelial cells adhesion by antibodies against L-selectin and CD18 reduced LTC_4 generation (14) showing for the first time the involvement of adhesion in transcellular eicosanoids biosynthesis.

PMN and platelets can physically interact at membrane level through specific adhesion molecules. P-selectin (15), previously known as Platelet Activation Dependent Granule External Membrane Protein (PADGEM) (16) or Granule Membrane Protein 140 (GMP-140) (17), is an integral glycoprotein of alpha granules maximally expressed on platelet surface after activation that recognizes components of PMN membrane that include the sialyl-Lewis X (neu5Ac α 2–3 Gal β 1–4 [Fuc α 1–3] Glc NAc β-R) and a protein (18–23). P-selectin-dependent platelet-leukocyte adhesion has been recently reported as the specific mechanism localizing PMN at the site of thrombus formation (24).

The aim of this study was to investigate the role of P-selectin-mediated PMN-platelet adhesion in TxB_2 and/or LTC_4 transcellular metabolism occurring between PMN activated by a specific agonist, such as fMLP, and platelets subsequently activated by PMN-released cathepsin G.

Materials and Methods

Chemicals

fMLP, prostaglandin (PG)E_1, PGE_2, TxB_2, N-2 hydroxyethyl piperazine-N 1-2-ethanesulfonic acid (HEPES), ethylene glycol-bis (b-aminoethyl ether)-N, N, N', N',-tetraacetic acid (EGTA), from Sigma Chemical Co. (St. Louis, MO); LTB_4, 6-trans-LTB_4, 6-trans-12-epi-LTB_4, LTC_4 and LTE_4 from Cayman Chemical Co. (Ann Arbor, MI). Cathepsin G purified from human PMN from Calbiochem (San Diego, CA); Dextran T500 and Ficoll-Hypaque from Pharmacia Fine Chemicals (Uppsala, Sweden); Triton X-100 from Aldrich Chimica S. r. l. (Milano, Italy) and purified human fibrinogen from Kabi Diagnostica

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(Stockholm, Sweden). Eglin C (recombinant CGP 32968) was kindly provided by Ciba Geigy (Basel, Switzerland).

5-[1,2-^{3}(H)] Hydroxytryptamine binoxalate (^{3}H-5-HT, specific activity 15–30 Ci/mmol); [5, 6, 8, 9, 11, 12, 14, 15, ^{3}H (N)]-TxB_2 (^{3}H-TxB_2), [5, 6, 8, 9, 11, 12, 14, 15-^{3}H-(N)]-arachidonic acid (^{3}H-AA), specific activity 180–240 Ci/mmol, were from du Pont de Nemours (Firenze, Italy).

fMLP and cytochalasin B were dissolved in DMSO at concentrations of 50 and 100 mM, respectively, stored at –20° C and diluted in isotonic saline just before use. Eglin C was dissolved in saline at concentration of 100 mg/ml just before use. Cathepsin G was dissolved in saline at concentrations of 20 μM and stored at –20° C until used.

Mouse anti P-selectin monoclonal antibodies GE12 ($F(ab)_2$ fragment) (24) and AC1.2 (16) were kindly provided by Dr. B. Furie (New England Medical Center Hospitals, Boston, MA).

Preparation of Washed PMN and Platelets

Blood was collected from healthy volunteers who had not received any medication for at least two weeks, anticoagulated with trisodium citrate (0.38% final concentration). Platelet-rich plasma (PRP) was prepared by centrifugation of whole blood at 200 ×g for 15 min. PMN were isolated from the remaining blood by Dextran sedimentation, followed by Ficoll-Hypaque gradient and hypotonic lysis of erythrocytes. PMN were washed and resuspended in Hepes Tyrode buffer (pH 7.4) containing: 129 mM NaCl, 9.9 mM $NaHCO_3$, 2.8 mM KCl, 0.8 mM KH_2PO_4, 0.8 mM $MgCl_2$-$6H_2O$, 5.6 mM Dextrose, 10 mM Hepes and 1 mM $CaCl_2$. Cellular suspensions contained ≥95% of PMN and an average of 1–2 platelet/100 PMN was usually observed.

Washed platelets were prepared by centrifuging PRP at 1,100 × g for 15 min after addition of 1 μM of PGE_1. The pellet was then resuspended in Hepes Tyrode containing1 μM PGE_1 and 5 mM EGTA and centrifuged at 1,100 × g for 10 min. Platelets were then resuspended in Hepes Tyrode at a concentration of 5 × 10^8/ml and kept at room temperature during the experiment.

Experimental Procedures

Platelets (10^8/ml) and PMN (10^7/ml) were incubated in a final volume of 1 ml of Hepes Tyrode (pH 7.4) containing 0.38 mg/ml fibrinogen and 2.5 μg/ml cytochalasin B in a Chrono-Log aggregometer for 2 min at 37° C under constant stirring at 1,000 rpm before addition of fMLP (1 μM). In the experiments in which anti-P-selectin antibody was used, GE12 was preincubated with platelets at a concentration of 30 μg/5 × 10^8 platelets/ml. Cell aggregation was recorded as increase in light transmission and expressed as percent of the maximal light transmission. Platelets were also activated by PMN-derived supernatants (30 s at 14,000 rpm in an Eppendorf centrifuge) prepared 1 min after fMLP stimulation (7). The reaction was stopped 3 or 30 min after addition of the stimulus, samples cooled to 0° C for 15 min and centrifuged in an Eppendorf centrifuge; supernatants were collected for further assays.

Cathepsin G activity in PMN supernatants was determined by continuous monitoring of the specific chromogenic substrate N-succinyl-Ala-Ala-Pro-Phe-p-nitroanilide (Sigma Chemical Co., St. Louis, MO) hydrolysis, as described (7).

Platelet serotonin (5-HT) release. Preparation of ^{3}H-5-HT-labeled platelets: PRP was incubated with 0.1 μCi/ml of ^{3}H-5-HT, at room temperature for 30 min. Platelets were then washed following standard procedure described before. Scintillation counting of 50 μl of PRP compared to 50 μl of platelet-poor plasma was carried out for measurement of uptake, which was about 90%. Release of radioactive 5-HT from ^{3}H-5-HT-labeled platelets was evaluated in supernatants of samples of platelets activated in mixed cell suspensions or by PMN-derived supernatants. The reaction was stopped by adding EGTA (5 mM f. c.) and paraformaldehyde (1% f. c.), followed by rapid centrifugation at 14,000 × g for 2 min. In preliminary experiments performed in the presence of imipramine (2.5 μM) to block 5-HT uptake, ^{3}H-5HT release was not different from that obtained in controls. For this reason in the experiments reported, imipramine was not used. ^{3}H-5-HT release was expressed as % of the total platelet content.

Cytofluorimetric Analysis

Unstimulated platelets (10^8/ml) and platelets activated by cathepsin G (10–200 nM) for 1 min at 37° C without stirring were fixed overnight in 1% paraformaldehyde. Fixed platelets were then washed three times, resuspended in Hepes Tyrode buffer and used for cytofluorimetric analysis. Fixed platelets were incubated with or without AC1.2 (ascites, 1:500 final dilution) for 30 min at room temperature, then washed twice in Hepes Tyrode. Samples were then incubated with anti-mouse FITC-conjugate IgG (20 μg/ml) for 30 min in the dark at room temperature, washed twice and resuspended in 500 μl of Hepes Tyrode buffer and analyzed by FACScan flow cytometer (FACSTAR, Beckton and Dickinson) storing data in list mode files. Determination of the percentage of platelets expressing P-selectin labeling was performed using a threshold set obtained with platelets treated only with anti-mouse FITC-conjugate IgG. Platelet mean P-selectin labeling was expressed as mean fluorescence in arbitrary units.

Determination of AA Metabolites Formation by Radioimmunoassay (RIA)

Supernatants from mixed cell suspensions or from platelets activated by PMN-derived supernatant, were ultrafiltered with Centricon 3 (Amicon) to remove proteins before RIA. TxB_2 was measured using an antiserum kindly provided by Prof. C. Patrono (G. D'Annunzio University, Chieti, Italy) (9). LTC_4 was quantified using a specific (1.6% cross reactivity with LTD_4 and 0.06% cross reactivity with LTE_4) commercial kit from Amersham Life Science (Amity S. r. l., Milano, Italy). The detection limit of RIAs was 50 and 80 pg/ml of incubate for TxB_2 and LTC_4, respectively. Values are reported as ng/ml of incubate.

Determination of 3H-AA Metabolites Formation by HPLC

Preparation of ^{3}H-AA-labeled PMN. Suspensions of PMN (3 × 10^7/ml) in Hepes Tyrode buffer were incubated (45 min, 37° C) with 0.25 μCi/ml of ^{3}H-AA, washed twice and resuspended in Tyrode buffer.

Preparation of ^{3}H-AA-labeled platelets. Washed platelets were resuspended (2 × 10^8 platelets/ml) in a Tris buffer (Tris 63 nM; NaCl 95 mM; KCl 5 mM; citric acid 12 mM, glucose 5.5 mM; fatty acid free bovine serum albumin 0.01%, pH 6.5) and incubated (45 min, 37° C) with 1 μCi/ml of ^{3}H-AA, washed twice in the presence of PGE_1 and suspended in Hepes Tyrode.

Preparation of samples. Experimental conditions and stimulation of samples of ^{3}H-AA-labelled PMN or ^{3}H-AA-labeled platelets were identical to those used with unlabeled cells. Reaction was stopped by addition of 2 volumes of iced acetone. Samples were kept at –20° C for 15 min, centrifuged at 3,000 rpm (30 min, at –4° C), the clear supernatants acidified to pH 4.5 with formic acid. Lipids were extracted twice with 2 volumes of chloroform and organic phases were evaporated under N_2 stream. Dried residues were dissolved in 100 μl of methanol: acetonitrile (1:1; vol:vol) immediately before HPLC analysis (2). The radioactivity recovered from samples through lipid extraction and HPLC was 59 ± 7% (mean ± SD; n = 3).

HPLC. The apparatus consisted of a liquid chromatograph (Beckman System Gold), equipped with a Diode Array Detector module 168 and a 5 μm reversed phase column (Nucleosil RP-18, 25 cm × 4.6 mm i. d., Chrompack, Mildebourg, The Netherlands). The methods used were modified from Powell (25) and Tordjman et al. (26).

Determination of ^{3}H-TxB_2. The mobile phase consisted of 50 mM Na_2HPO_4:CH_3CN (62.5:37.5; vol:vol, pH 5.1). Elution was performed at isocratic conditions in a single run of 30 min (flow rate 1 ml/min. Standards and samples were revealed at 205 nm. ^{3}H-TxB_2 was used as authentic standard. The eluate, collected in fractions of 24 s each, was counted for radioacivity. In platelet/PMN samples activated in the absence of GE12 (control), the radioactivity eluted with the same retention time (16 min) of authentic ^{3}H-TxB_2 was 28.0 ± 7.4% (mean ± S. D.; n = 3) of total radioactivity eluted from HPLC (9).

Determination of ^{3}H-LTC_4. Immediately before HPLC injection, authentic standard of LTC_4 (100 ng) was added to samples. The mobile phase consisted

Table 1 TxB_2 and LTC_4 production by mixed cell suspensions and cathepsin G release by PMN challenged with different concentrations of fMLP

fMLP M	TxB_2 ng/ml	LTC_4 ng/ml	Cathepsin G nM
–	0.9	<0.8	<10
10^{-9}	1.0	<0.8	<10
10^{-8}	1.1	<0.8	30
10^{-7}	13.0	0.93	139
10^{-6}	32.4	1.72	248

Data are means of two different experiments performed in duplicate. Cells (10^8 platelets/10^7 PMN/ml for TxB_2 and LTC_4 production and 10^7 PMN/ml for cathepsin G release) were stimulated by fMLP in the presence of 2.5 μg/ml cytochalasin B and 0.38 mg/ml fibrinogen. Stimulus was added after 2 min stirring at 37° C and the reaction stopped at 3 min. For further details see Materials and Methods.

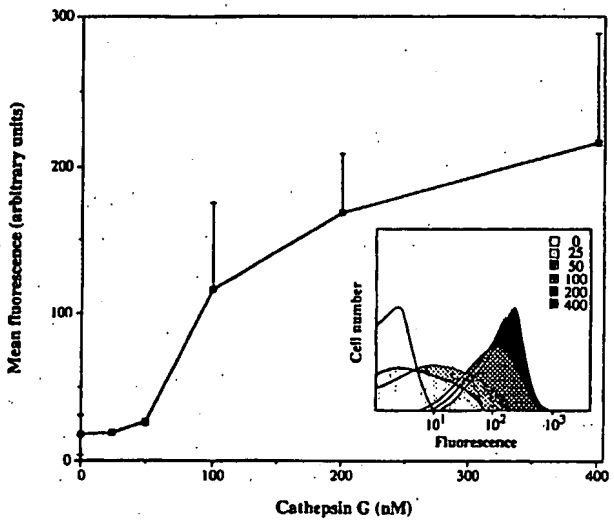

Fig. 1 P-selectin expression on cathepsin G-activated platelets. P-selectin-dependent fluorescence of platelets challenged with increasing concentrations of cathepsin G was determined by FACS (see Materials and Methods). Mean platelet fluorescence is expressed in arbitrary units (mean and SD of 3 experiments). P-selectin expression by thrombin-activated platelets was measured for comparison. Platelets activated with 0.5 U/ml of thrombin express mean fluorescence corresponding to 157 ± 84 arbitrary units (mean ± SD; n = 5). The insert shows representative tracings of cytofluorimetric analysis of platelets activated by different concentrations of cathepsin G (0, 25, 50, 100, 200, 400 nM)

of: CH_3OH:CH_3COOH 0.1%:CH_3CN (56.5: 33: 10.5: vol: vol: vol). Elution was performed at isocratic conditions in a single run of 30 min (flow rate 0.5 ml/min). The absorbance of the column effluent was monitored at 280 nm, and UV spectra were recorded every 2 s. The eluate, collected in fractions of 24 s each, was counted for radioactivity determination. The radioactivity eluted with the same retention time (9.4 min) of the peak showing the UV absorption spectrum of standard LTC_4 was considered as ^{3}H-LTC_4. In platelet/PMN samples activated in the absence of GE12 (control) the radioactivity identified as ^{3}H-LTC_4 corresponded to 22.7 ± 9.5% (mean ± S. D.; n = 3) of total radioactivity eluted from HPLC.

Statistical Analysis

Data, reported as means and S. D., were analyzed by paired Student's-t-test. Medians with 25°–75° percentile (ptc) were reported, when the sample distribution was not normal. In this case, statistical analysis has been performed by Wilcoxon signed-rank test.

Results

TxB_2 and LTC_4 Production in PMN/Platelet Mixed Cell Suspensions Challenged with fMLP: Role of Cathepsin G

Under the experimental conditions used in this study, PMN/platelet mixed cell suspensions challenged with fMLP produced average amounts of 37.1 ng/ml of TxB_2 (median = 30.7; 23.3–49.2; 25°–75° ptc; n = 13) and of 2.1 ng/ml of LTC_4 (median = 2.4; 0.8–2.7; 25°–75° ptc; n = 17). When platelets were challenged with supernatants of activated PMN, TxB_2 production was reduced to about 50% of that produced by mixed cell suspensions, according with previous results (9), while LTC_4 was below the detection limit of the assay (80 pg/ml). PMN and platelets challenged alone with fMLP did not produce detectable amounts of either metabolite. In few experiments PMN alone produced detectable LTC_4, attributed to the presence of high number of basophils and eosinophils in the PMN preparation, which can indeed generate LTC_4 by themselves. These experiments were not considered in the final evaluation.

TxB_2 and LTC_4 production in mixed cell suspension was dependent on the concentration of fMLP added and correlates with the amount of cathepsin G released by PMN challenged with the same concentration of agonist (Table 1).

Similarly to what already observed in respect to TxB_2 transcellular metabolism in this system (9), the importance of cathepsin G-induced platelet activation on LTC_4 production was also shown. LTC_4 production was measured by RIA in mixed cell suspensions activated by fMLP in the absence or the presence of 1 mg/ml of eglin C, a cathepsin G inhibitor. Eglin C significantly ($p = 0.001$, by Wilcoxon test) reduced LTC_4 from average control of 3.10 ng/ml (median 2.65; 0.65–3.50, 25°–75° ptc) to 0.98 ng/ml in eglin C-pretreated samples (median 0.25; 0.10–1.40, 25°–75° ptc), indicating that cathepsin G has an important role in this PMN/platelet cooperation. Eglin C neither modified AA-induced platelet TxB_2 production (9) nor fMLP-induced LTB_4 production by PMN (not shown).

P-Selectin Expression by Cathepsin G-Activated Platelets and Inhibition of Mixed Cell Aggregation by Anti-P-Selectin Antibody

As reported in Fig. 1, cathepsin G at concentrations in the range of those released by fMLP-activated PMN (7) stimulated P-selectin expression on platelet surface in a concentration-dependent manner.

fMLP-induced mixed cell aggregation was significantly reduced by GE12, a $F(ab)_2$ fragment of a monoclonal antibody against P-selectin, platelet 5-HT release being unchanged (Fig. 2, panel A). This antibody did not inhibit homologous platelet aggregation and platelet 5-HT release stimulated by PMN-derived supernatants (Fig. 2, panel B). GE12 did not affect fMLP-induced PMN homologous aggregation (not shown).

These data indicate that the inhibitory effect of GE12 on mixed cell aggregation is due to the inhibition of PMN-platelet adhesion mediated by P-selectin, maximally expressed on platelets activated by PMN-derived cathepsin G.

Role of P-Selectin-Mediated PMN/Platelet Adhesion in TxB_2 and LTC_4 Transcellular Metabolism

Production of TxB_2 and LTC_4 in mixed cell suspensions stimulated by fMLP in the absence or presence of GE12 was evaluated by specific RIAs. Synthesis of ^{3}H-TxB_2 in ^{3}H-AA-labeled PMN/unlabeled platelets and ^{3}H-LTC_4 in unlabeled PMN/^{3}H-AA-labeled platelets, challeng-

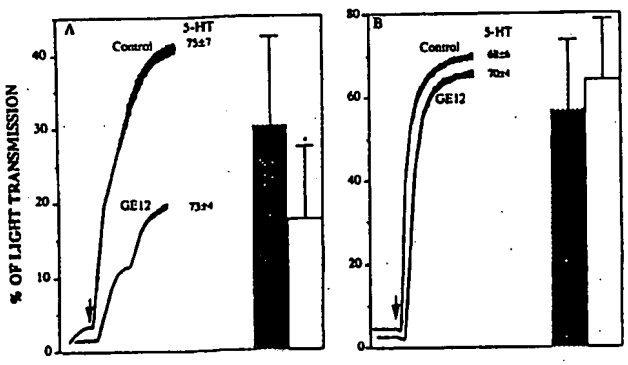

Fig. 2 Inhibition by GE12 of mixed PMN/platelet aggregation. fMLP-induced PMN/platelet aggregation (panel A) and platelet aggregation induced by PMN-derived supernatant (panel B) in the absence (white bars) and in the presence (black bars) of GE12. Platelets (10^8/ml) and PMN (10^7/ml) were incubated in the presence of fibrinogen (0.38 mg/ml) and cytochalasin B (2.5 μg/ml). Arrows indicate addition of fMLP (1 μM) to mixed cell suspensions (panel A); supernatant (30 s at 14,000 rpm in Eppendorf centrifuge) from PMN activated with 1 μM fMLP for 1 min in the presence of fibrinogen (0.38 mg/ml) and cytochalasin B (2.5 μg/ml) was added (arrows) immediately after preparation to platelets preincubated at 37° C (panel B). The cellular aggregation was measured as percent of light transmission and reported both as representative curves and as bars indicating the percentage of light transmission at 3 min after addition of the stimulus (means and SD; n = 15 and n = 5 for panel A and B, respectively). *p = 0.01 by Wilcoxon test. 5-HT release (percent of total content) in the same samples is reported as means ± S. D.

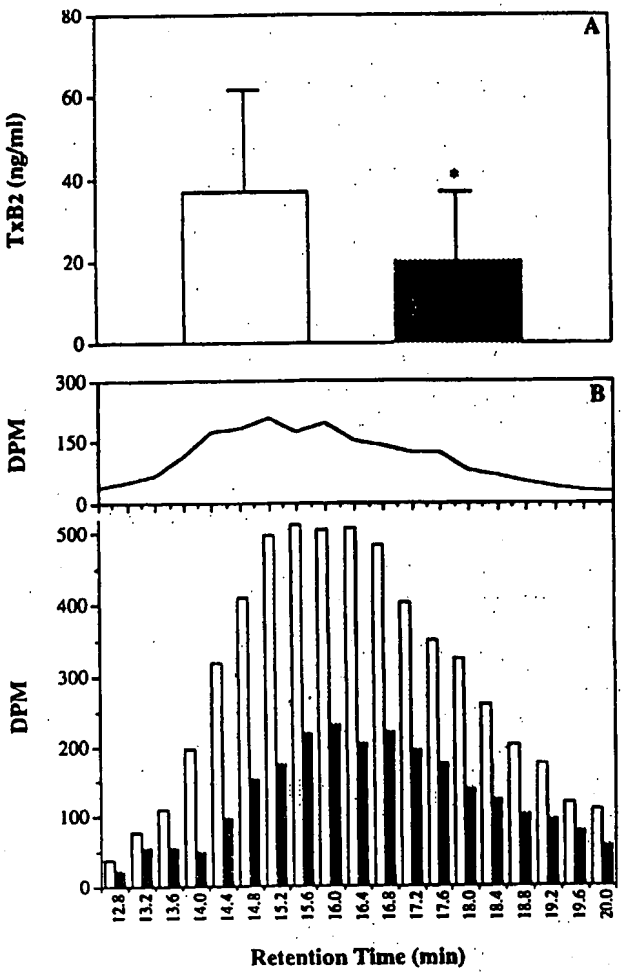

Fig. 3 Inhibition by GE12 of TxB_2 production in mixed cell suspensions challenged with fMLP.
Mixed cell suspensions of platelets (10^8/ml) and PMN (10^7/ml) were incubated in the presence of fibrinogen (0.38 mg/ml) and cytochalasin B (2.5 μg/ml) 2 min before stimulation with fMLP (1 μM). Panel A: TxB_2 production (measured by RIA) was determined in fMLP-stimulated PMN-platelet suspensions in the absence (white bars) and in the presence (black bars) of GE12. Reaction was stopped 3 min after stimulus, samples centrifuged and proteins removed by filtration. Figures are means and S. D. of 13 different experiments and are significantly different (*p = 0.01 by Wilcoxon test). Panel B: ^{3}H-TxB_2 production (measured by HPLC) was determined in fMLP-stimulated ^{3}H-AA-labeled PMN/unlabeled platelet suspensions in the absence (white bars) or in the presence (black bars) of GE12. Reaction was stopped by addition of 2 volumes of iced acetone. Samples were kept at –20° C for 15 min, centrifuged at 3,000 rpm for 30 min at –4° C, supernatants were acidified to pH 4.5 with formic acid, lipids extracted twice with 2 volumes of chloroform and organic phases evaporated under N_2 stream. Dried residues were dissolved in 100 μl of methanol: acetonitrile (1:1, vol:vol) and injected into HPLC. Bars indicate the radioactivity contained in the fractions corresponding to the retention time (16 min) of the peak of standard ^{3}H-TxB_2 (top of panel B) and are representative of 6 different experiments. Radioactivity identified as TxB_2 was significantly (p <0.001 by paired Student's-t-test) reduced by 63 ± 12% in samples incubated in the presence of GE12 in respect to controls. For further details see Materials and Methods and Results

ed with fMLP in the absence or presence of GE12, was evaluated by HPLC.

TxB_2 production in mixed cell suspensions stimulated for 3 min by fMLP was significantly (p = 0.01 by Wilcoxon test) reduced from mean 37.1 ng/ml in controls (median = 30.7; 23.3–49.2, 25°–75° ptc; n = 13) to 20.7 ng/ml in the presence of GE12 (median = 16.4; 8.9–27.5, 25°–75° ptc; n = 13; Fig. 3, panel A). A similar inhibitory effect was observed in samples stimulated for 30 min: 66.6 ± 10.9 and 44.4 ± 9.1 (means ± SD, n = 3) ng/ml of TxB_2 were measured in the absence or in the presence of GE12, respectively.

According with previous data (9), when ^{3}H-AA-labelled PMN/unlabeled platelets were challenged with fMLP, ^{3}H-TxB_2 was formed. Tracings related to TxB_2 are scattered over a wide retention time, both in biological and standard radioactive samples, as previously observed also with non radioactive standard (9). The HPLC profile of radioactivity obtained with ^{3}H-labeled PMN after fMLP challenge in the absence of platelets did not show any peak with the retention time of TxB_2. Radioactivity identified as ^{3}H-TxB_2 in ^{3}H-AA-labeled PMN/unlabeled platelets was significantly reduced (p <0.001, n = 6) to 37 ± 12% (mean ± S. D.) of the control (Fig. 3, lower panel B). In contrast, the antibody did not modify TxB_2 synthesis by platelets activated by PMN-derived supernatants (24.3 ± 7.5 ng/ml in the absence versus 22.1 ± 15.0 ng/ml in the presence of the antibody; n = 3).

Similarly to TxB_2, LTC_4 production measured at 3 min after stimulation was significantly (p = 0.01 by Wilcoxon test, n = 17) reduced from 2.11 ng/ml (median = 2.4; 0.8–2.7, 25°–75° ptc) in control samples to 1.07 ng/ml in GE12-treated samples (median = 1.0; 0.3–1.7, 25°–75° ptc; Fig. 4, panel A). LTC_4 measured in 30 min-stimulated PMN-platelet suspensions remained reduced in GE12-treated samples in respect to the controls (0.96 ± 0.03 versus 2.11 ± 0.08 ng/ml; means ± SD, n = 3). HPLC analysis of radioactivity showed synthesis of ^{3}H-LTC_4 in samples of unlabeled PMN/^{3}H-AA-labeled platelets challenged with fMLP, but not in samples of ^{3}H-AA-labeled platelets challenged with supernatant from fMLP-activated PMN. The radioactivity identified as ^{3}H-LTC_4 was reduced to 31.5 ± 17.0% of control (p <0.001; n = 3) in GE12-treated samples (Fig. 4, panel b).

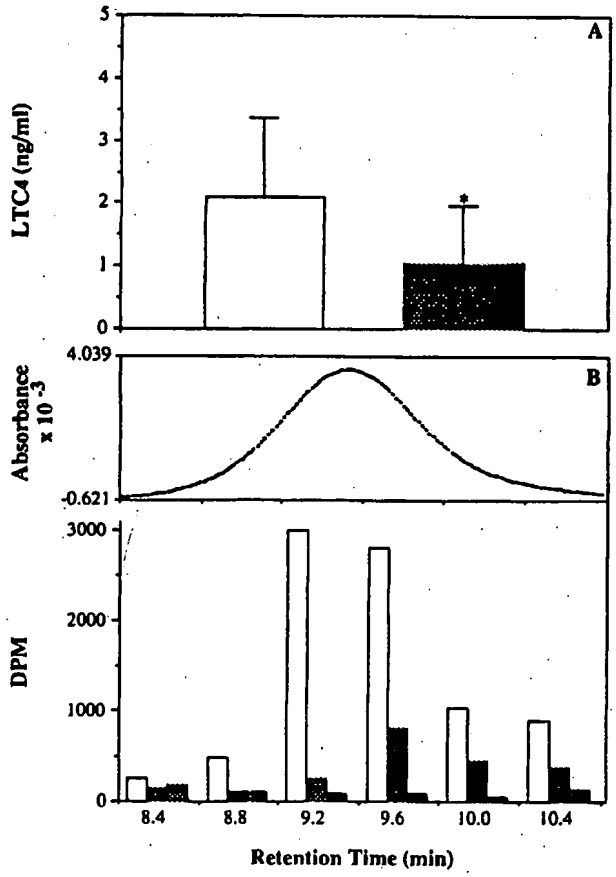

Fig. 4 Inhibition by GE12 of LTC_4 production in mixed cell suspensions challenged with fMLP. Mixed cell suspensions of platelets (10^8/ml) and PMN (10^7/ml) were treated as described in Fig. 3. Panel A: LTC_4 production (measured by RIA) was determined in fMLP-stimulated PMN/platelet suspensions in the absence (white bars) or in the presence (black bars) of GE12. Figures are means and S. D. of 17 different experiments performed in triplicate and are significantly different (*p = 0.01 by Wilcoxon test).
Panel B: ^{3}H-LTC_4 production (measured by HPLC) was determined in fMLP-stimulated unlabeled PMN/^{3}H-AA-labeled platelet suspensions in the absence (white bars) or in the presence (black bars) of GE12 and in ^{3}H-AA-labeled platelet suspensions challenged with supernatants from fMLP-activated PMN (grey bars). Reaction was stopped and samples treated as described in Fig. 3. Dried residues were dissolved in 100 µl of methanol: acetonitrile (1:1; vol:vol) and authentic LTC_4 was added to radioactive samples. Bars indicate radioactivity identified as ^{3}H-LTC_4 in the fractions eluted with the same retention time (9.4 min) of the peak of authentic LTC_4 (top of panel B). Bars are representative of 3 different experiments. Radioactivity corresponding to LTC_4 was significantly ($p < 0.001$ by paired Student's t-test) reduced by 68.5 ± 17.3% in the presence of GE12 in respect to control. For further details see Materials and Methods and Results

Release of AA, separated by TLC (9), from ^{3}H-AA-labeled cells (platelets or PMN) in mixed cell suspensions challenged with fMLP was not significantly modified by prevention of PMN/platelet adhesion (not shown).

In preliminary experiments the anti-P-selectin monoclonal antibody AC1.2, devoid of function inhibitor effect (16, 28), did not reduce TxB_2, nor LTC_4 synthesis in mixed cell suspensions (not shown).

These data indicate that the inhibitory effect of GE12 on TxB_2 and LTC_4 production in mixed cell populations depends on the prevention of AA and LTA_4 exchange between PMN and platelets as a consequence of the inhibition of P-selectin-dependent cell-cell adhesion.

Discussion

In the experimental model used in this study PMN, specifically activated by fMLP, release cathepsin G, that subsequently stimulates platelet aggregation, AA metabolism, release reaction of dense bodies (4, 6–9).

After addition of fMLP to mixed cell populations a tight membrane to membrane adhesion between PMN and platelets was observed at electron microscopy during the formation of giant mixed aggregates (7). In a previous study (9) we reported that in this system AA may be transferred from PMN to platelet increasing platelet TxB_2 production and suggested a possible role of cell adhesion in modulating this one-way phenomenon. In the present study, we have preliminarily extended this observation to the bidirectional transcellular metabolism of LTC_4. In agreement with recent data (11, 12), we have shown that ^{3}H-LTC_4 was produced in mixed unlabeled PMN/^{3}H-AA-labeled platelet population stimulated by fMLP, indicating a double exchange: AA transfer from cathepsin G-activated platelets to fMLP-activated PMN and subsequent LTA_4 transfer from PMN to platelets. LTA_4 is transformed in LTC_4 by platelet glutathione-S-transferase (10, 27).

It has been suggested that P-selectin-mediated PMN-platelet adhesion may result in the formation of a sequestered microenvironment between cell membranes (28). This concept prompted us to investigate the possible role of P-selectin in the production of TxB_2 and LTC_4 resulting from AA transcellular metabolism between adhering PMN and platelets.

In the experimental conditions used to study AA metabolism by PMN-platelet mixed suspensions, cathepsin G was able to increase P-selectin expression on platelet surface. The use of an antibody against P-selectin (GE12) in fMLP-treated PMN/platelet suspensions substantially reduced mixed cell aggregation, despite cathepsin G release from PMN (28) or platelet activation (monitored as serotonin release) were not modified.

The prevention of P-selectin-mediated PMN/platelet adhesion by the antibody GE12 resulted in a significant reduction of immunologically reactive TxB_2 synthesis. In parallel, ^{3}H-TxB_2 in ^{3}H-AA-labeled PMN/unlabeled platelets was also reduced by the inhibition of P-selectin-mediated PMN/platelet adhesion. This indicates that AA transfer from activated PMN to platelets, contributing to TxB_2 synthesis, may be modulated by P-selectin-dependent cell-cell contact.

Similarly, immunologically reactive LTC_4 production was strongly reduced by GE12 as well as the formation of ^{3}H-LTC_4 in mixed samples of unlabeled PMN/^{3}H-AA-labeled platelets stimulated with fMLP.

As already reported for TxB_2 synthesis (9), cathepsin G-mediated platelet activation was also an essential step for LTC_4 transcellular metabolism in this experimental model as indicated by the inhibitory effect of eglin C, a cathepsin G inhibitor, on LTC_4 production.

Activated platelets represent an important source of free AA which is only partially utilized by platelets themselves to synthesize 12-lipoxygenase and cyclooxygenase metabolites. Part of AA from activated platelets can be utilized by adjacent activated PMN via 5-lipoxygenase. This is a cytosolic enzyme in resting PMN and is rapidly translocated to membrane after activation (29); this position would favor the utilization of AA provided by platelets and the export of LTA_4 to platelets for further metabolism.

In this way platelets significantly contribute to the synthesis of LTA_4, the precursor of LTB_4 in PMN (30, 31) and of LTC_4 (11, 12) and lipoxins in platelets (11, 32).

Inhibition of cathepsin G (the major platelet agonist in this model) not only prevents AA release from platelets, that contribute to LTA_4 synthesis, but also P-selectin expression, that is required for optimal AA cooperative metabolism.

LTA_4, produced by activated PMN, can be transformed to LTC_4 by platelet glutathione-S-transferase or to lipoxins by 12-lipoxygenase or, when not taken up by platelets, non enzymatically converted into LTB_4-isomers.

A full understanding of the mechanism(s) by which prevention of cell-cell adhesion reduces LTC_4 production, would require further studies taking into account all these metabolites. However, the most probable explanation for the GE12-dependent inhibition of LTC_4 is that, by preventing P-selectin-mediated membrane to membrane contact, optimal conditions are removed for transfer of both AA from platelet to PMN and LTA_4 from PMN to platelet.

This conclusion is also supported by the recent observation that PMN-endothelial cell LTC_4 transcellular metabolism is reduced by blocking cell-cell adhesion (14). Our study extends this previous observation, being, at the best of our knowledge, the first report showing that PMN-platelet interaction via P-selectin plays a role in LTC_4 cooperative synthesis.

LTC_4 and TxB_2 are two of the most potent vasoconstrictors, produced during PMN/platelet interactions. Recently leukocyte accumulation at the site of platelet thrombus was shown to be prevented in vivo by anti P-selectin antibody (24). This observation supports the hypothesis that P-selectin-dependent PMN/platelet adhesion could be a mechanism localizing PMN/platelet metabolic interaction in vivo. However, very few data (14) are available at the moment demonstrating the obvious hypothesis that cell-cell adhesion through specific adhesion molecules could create optimal conditions for metabolic cooperation. AA transcellular metabolism is a well known cooperative phenomenon probably playing a significant biological role in several physiopathological situations including inflammatory, pulmonary and cardiovascular diseases (33).

In conclusion, when under suitable conditions PMN are activated by fMLP and platelets by subsequently PMN-released cathepsin G, stable PMN/platelet contact occurs which is, at least in part, a consequence of the expression of the specific adhesion molecule P-selectin. Although we cannot exclude a role for other possible adhesive proteins, such as fibrinogen (34) and thrombospondin (35), data reported in this paper indicate for the first time that P-selectin-mediated PMN-platelet contact may facilitate the transcellular metabolism of AA resulting in increased production of TxB_2 and LTC_4.

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Differential Roles of Selectins and the α_4-Integrin in Acute, Subacute, and Chronic Leukocyte Recruitment In Vivo[1]

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Adhesion blocking mAbs specific for rat P-, E-, and L-selectin and the α_4-integrin were used to characterize leukocyte recruitment mechanisms in models of LTC_4 (acute), LPS (subacute), and adjuvant-induced (chronic) inflammation. Intravital microscopy was employed to measure leukocyte rolling and adhesion in rat mesenteric venules. Superfusing the mesentery with 20 nM LTC_4 elicited an increase in leukocyte rolling (66.8 ± 3.8 vs 18.2 ± 3.2 cells/min control) that was completely eliminated by an anti-rat P-selectin mAb. Superfusion with 1 μg/ml LPS induced a significant increase in leukocyte rolling within 15 min (73 ± 8 vs 33 ± 6 cells/min control). Rolling increased further starting at 105 min and peaked by 150 min (141 ± 23 cells/min). LPS-induced leukocyte rolling was eliminated during the first 90 min by the P-selectin mAb. The later increase in leukocyte rolling was not prevented by a second treatment with P-selectin mAb or a function-blocking mAb against rat E-selectin. This later phase of leukocyte rolling was blocked by treatments with mAbs against either the α_4-integrin or L-selectin. Twelve days following *Mycobacterium butyricum* immunization, 300 to 500 rolling cells/min were observed. This could be reduced ~50 to 60% by mAb against either the α_4-integrin or L-selectin. The combination of both mAbs eliminated ~90% of rolling. Neither the P- nor E-selectin mAbs reduced rolling in this chronic inflammatory model. This study highlights differences in leukocyte adhesive mechanisms elicited by different stimuli and at different time points within the same vascular bed. ***The Journal of Immunology,*** **1997, 159: 4514–4523.**

The recruitment of leukocytes from the blood to the extravascular space is an important defensive response to foreign pathogens and tissue injury, but has also been implicated in the pathology of various inflammatory disease states. A sequential cascade of leukocyte-endothelial cell adhesive interactions appears to be essential for the efficient recruitment of leukocytes to sites of inflammation (1–6). This multistep process is initiated by the selectin family of adhesion molecules ($CD62^L$, $CD62^P$, and $CD62^E$), which tether leukocytes to the endothelium and mediate weak transient interactions that manifest as leukocyte rolling. Rolling leukocytes may then adhere firmly to the endothelium via β_2-integrins (CD11/CD18) and emigrate from the vessel. More recently an alternative recruitment pathway has been characterized in which the α_4-integrin ($\alpha_4\beta_1$ and $\alpha_4\beta_7$) can mediate both the rolling and adhesion steps of the recruitment cascade (7–13). Human lymphocytes will tether, roll, and adhere via the α_4-integrin under laminar flow conditions in vitro (8, 9, 11). Leukocytes will also roll and adhere in vivo via the α_4-integrin (12).

Although there have been many studies characterizing the roles of leukocyte adhesion molecules in the recruitment of inflammatory cells, the role of each adhesion molecule in different types of inflammation is unclear. Studies have been conducted in different species (rat, mouse, cat, rabbit, human) and tissues (mesentery, skeletal muscle, lymph node, liver), using different reagents (Abs, drugs), and different protocols (myeloperoxidase assay, radiolabeled cells, intravital microscopy). These factors have made it difficult to ascertain the role of the various adhesion molecules in different inflammatory situations within the same microvascular bed, and to evaluate the universality of the current leukocyte recruitment paradigm.

The objective of this study was to systematically characterize the role of the selectins and the α_4-integrin in mediating leukocyte recruitment under short-term acute (1 h), longer-term subacute (3 h), and chronic (12 d) inflammatory conditions. Intravital microscopy was used to examine the adhesion molecules mediating leukocyte-endothelial cell interactions in rat mesenteric postcapillary venules during: 1) acute inflammation induced by LTC_4; 2) subacute inflammation using an LPS-induced model; and 3) chronic inflammation with vasculitis following immunization with *Mycobacterium butyricum*. Novel Abs developed in selectin-deficient mice against P-selectin and E-selectin were used in this study as well as previously described Abs against L-selectin and the α_4-integrin. The findings of this study demonstrate significant differences in the contributions of P-, E-, and L-selectin and the α_4-integrin to leukocyte rolling and adhesion under different inflammatory conditions in the same vascular bed. Additionally, the data raise the possibility that another molecule exists that may recruit rolling leukocytes under baseline conditions and in chronically inflamed microvessels.

Materials and Methods

Intravital microscopy

Male Sprague-Dawley rats (160–220 g) were maintained on a purified laboratory diet and fasted for 18 to 24 h before surgery. Animals were anesthetized with an i.p. injection of sodium pentobarbitol (55 mg/kg body

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weight). The right carotid artery was cannulated to measure systemic arterial blood pressure (model P23XL pressure transducer; Viggo-Spectramed, Oxnard, CA; and model 7 physiologic recorder; Grass Instruments Co., Quincy, MA), while the right jugular vein was cannulated to administer Abs and maintain anesthetic. Following laparotomy, rats were placed in the supine position on an adjustable Plexiglass microscope stage and a segment of the mid-jejunum was exteriorized and prepared for intravital microscopy as previously described (12, 14–16).

The mesenteric preparation was observed through an intravital microscope (Optiphot-2, Nikon Inc.; Mississauga, Canada) with a ×25 objective lens (Wetzlar L25/0.35; E. Leitz Inc., Munich, Germany) and a ×10 eyepiece. A video camera (model 5100 HS; Panasonic, Osaka, Japan) mounted on the microscope projected the image onto a color monitor (model PVM 2030; Sony, Tokyo, Japan), and the images were recorded using a videocassette recorder (model AG-1790; Panasonic) for subsequent playback analysis. The final magnification of the image on the monitor was ×1800. Single unbranched mesenteric venules (25–50 μm in diameter) were selected for study. The same section of venule was observed throughout the experiment to control for variations between different regions. Venular diameter was measured on-line using a video caliper (Microcirculation Research Institute, Texas A&M University, College Station, TX). Centerline RBC velocity was also measured on-line using an optical Doppler velocimeter (Microcirculation Research Institute, Texas A&M University).

The number of rolling and adherent leukocytes was determined off-line during video playback analysis. Leukocytes were considered adherent to the venular endothelium if they remained stationary for a period of time equal to or exceeding 30 s. Rolling leukocytes were defined as those white blood cells that moved at a velocity less than that of erythrocytes within the same vessel. The flux of rolling leukocytes was determined as the number of white blood cells that rolled past a fixed point in the venule during a 1-min interval using frame-by-frame analysis. Leukocyte rolling velocity was calculated from the average time required for 15 randomly selected leukocytes to travel along a 100-μm venular segment. Leukocyte emigration was measured as the number of extravascular leukocytes observed within the field of view (275 × 190 μm).

Monoclonal Abs

New function-blocking Abs to rat P-selectin (RMP-1, IgG_{2a} isotype) and rat E-selectin (RME-1, IgG_1 isotype) were examined in this study. These Abs bind to rat P-selectin and E-selectin, respectively, and block leukocyte adhesion to these ligands in vitro (17, 18). In preliminary experiments, the optimal concentration for RMP-1 in vivo was 0.5 mg/rat i.v. (2.0–2.5 mg/kg). The anti-rat E-selectin mAb (RME-1) was also used in our experiments at 0.5 mg/rat i.v. (2.0–2.5 mg/kg). The anti L-selectin mAb HRL-3 ($F(ab)_2$ fragments) was used at 1 mg/kg i.v. as previously reported (12). The anti-α_4-integrin mAb TA-2 (IgG_1 isotype) was used at 4 mg/kg i.v. as previously reported (12). A nonblocking anti-rat P-selectin mAb RP-2 (IgG_1 isotype) was used as a control (2.5 mg/kg i.v.). These Abs were used at optimal concentrations based on previous dose-response studies.

LTC_4-induced leukocyte recruitment

We have previously established a model of low baseline leukocyte trafficking to examine acute leukocyte recruitment in vivo (14–16). Some preparations were superfused continuously with 20 nM LTC_4 (Cayman Chemical Co., Ann Arbor, MI) following an initial 5-min baseline recording. This protocol has been shown previously to rapidly increase leukocyte trafficking through mesenteric venules (15). Fifteen minutes after initiating LTC_4 superfusion, animals were treated i.v. with Abs against P-selectin, E-selectin, or the α_4-integrin. Leukocyte trafficking was followed for 45 min after Ab administration.

LPS-induced leukocyte recruitment

Control preparations revealed little or no change in hemodynamic parameters or leukocyte kinetics over a 180-min experiment. After an initial 5-min baseline recording, some preparations were superfused with buffer containing 1 μg/ml LPS (*Escherichia coli* serotype 0127:B8; Sigma Chemical Co., St. Louis, MO). This dose of LPS has been shown previously to elicit leukocyte recruitment in vivo (19). Function-blocking Abs were used to characterize the adhesion molecules mediating LPS-induced leukocyte recruitment in this model. Rats were treated i.v. with anti-P-selectin mAb (RMP-1) 1 min before superfusion with LPS. This treatment was repeated at 105 min to ensure neutralization of P-selectin. Other groups were treated at 105 min with anti-E-selectin mAb (RME-1), anti-L-selectin mAb (HRL-3), or a mAb against the α_4-integrin (TA-2). The effects of Ab treatments on leukocyte trafficking were examined over the 180-min treatment protocol.

Adjuvant-induced vasculitis

Under light anesthetic (diethyl ether; BDH Inc., Toronto, Canada), male Sprague-Dawley rats (160–220 g) were injected s.c. at the base of the tail with a solution of heat-killed *Mycobacterium butyricum* (Difco Laboratories, Detroit, MI) in Freund's mineral oil adjuvant (Difco) (0.75 mg of *M. butyricum* in 0.1 ml of adjuvant). Previous experiments using intravital microscopy revealed a tremendous increase in leukocyte trafficking through mesenteric postcapillary venules 4 to 20 days after immunization (12, 20). Twelve days after immunization, leukocyte trafficking was measured during the first 20 min following exteriorization of the mesentery to establish baseline recruitment. Animals were then treated at 20 min with Abs against P-selectin (RMP-1), E-selectin (RME-1), L-selectin (HRL-3), and/or the α_4-integrin (TA-2). The effects of Ab treatment on leukocyte rolling flux and leukocyte adhesion were measured over the next 45 min.

Statistical analysis

All values are reported as means ± SEM. The data within groups were compared using a paired Student's *t*-test with Bonferroni corrections for multiple comparisons where appropriate. An unpaired Student's *t*-test was used to compare between groups. Statistical significance was set at $p < 0.05$.

Results

RMP-1 blocks P-selectin-dependent rolling

As LTC_4-dependent leukocyte rolling has previously been shown to be P-selectin dependent (15), we used this model to demonstrate that the novel mAb RMP-1 blocks P-selectin-dependent leukocyte recruitment. Figure 1 demonstrates the effects of 20 nM LTC_4 superfusion on leukocyte-endothelium interactions in rat mesenteric venules. Leukocyte rolling flux was significantly increased within 15 min of LTC_4 superfusion (Fig. 1*A*). This increase in leukocyte rolling was not affected by a nonblocking P-selectin mAb (RP-2), but was completely reversed by a blocking anti-P-selectin mAb (RMP-1). However, P-selectin blockade did not reduce leukocyte rolling flux below initial baseline levels (Fig. 1*A*). Administration of RMP-1 to control animals also failed to reduce leukocyte rolling below baseline, even with higher concentrations of Ab (data not shown).

In Figure 1*B*, it can be seen that LTC_4 treatment caused a reduction in leukocyte rolling velocity that was not affected by the nonblocking mAb RP-2. This reduction in leukocyte rolling velocity was completely reversed by the blocking P-selectin mAb RMP-1 (Fig. 1*B*). The blocking anti-P-selectin mAb also reversed LTC_4-induced increases in leukocyte firm adhesion (Fig. 1*C*). This treatment likely did not reduce adhesion directly, but rather prevented further recruitment by blocking the prerequisite leukocyte rolling necessary for firm adhesion.

The effects of other Ab treatments on LTC_4-induced leukocyte recruitment are summarized in Figure 2. Similar to the treatment with nonblocking anti-P-selectin mAb, blocking mAbs directed against E-selectin (RME-1) or the α_4-integrin (TA-2) did not affect LTC_4-induced increases in leukocyte rolling flux or adhesion, or the reduction in leukocyte rolling velocity. Although the treatments are only represented at the 30-min time point, these Abs did not have any effects at other time points (data not shown). We have previously demonstrated that the L-selectin mAb (HRL-3) does not affect LTC_4-induced leukocyte recruitment (15).

Early LPS-induced leukocyte recruitment is P-selectin dependent

The effects of LPS superfusion on leukocyte recruitment over a 180-min time period are shown in Figure 3. Leukocyte rolling flux was significantly increased by 15 min after initiation of

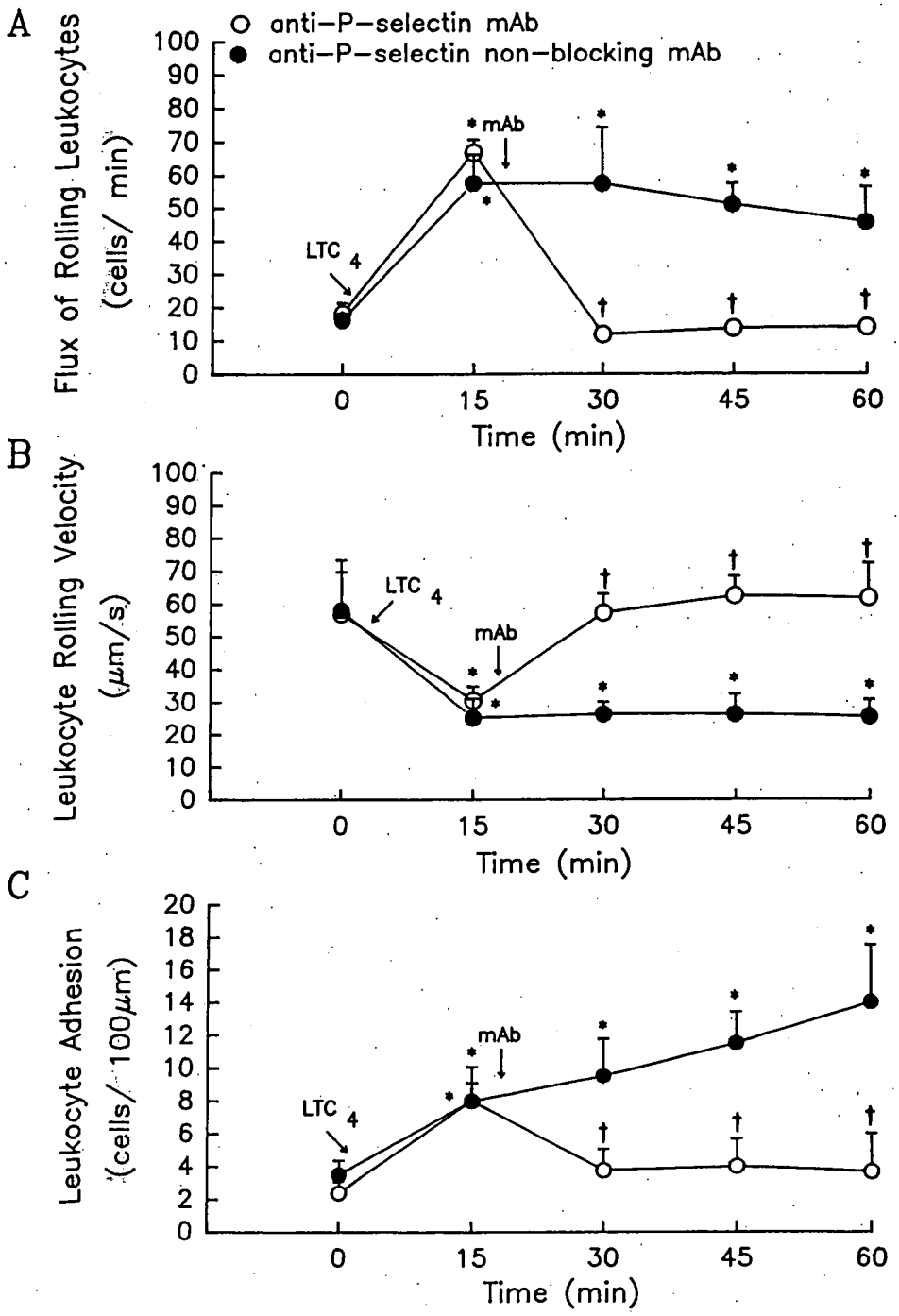
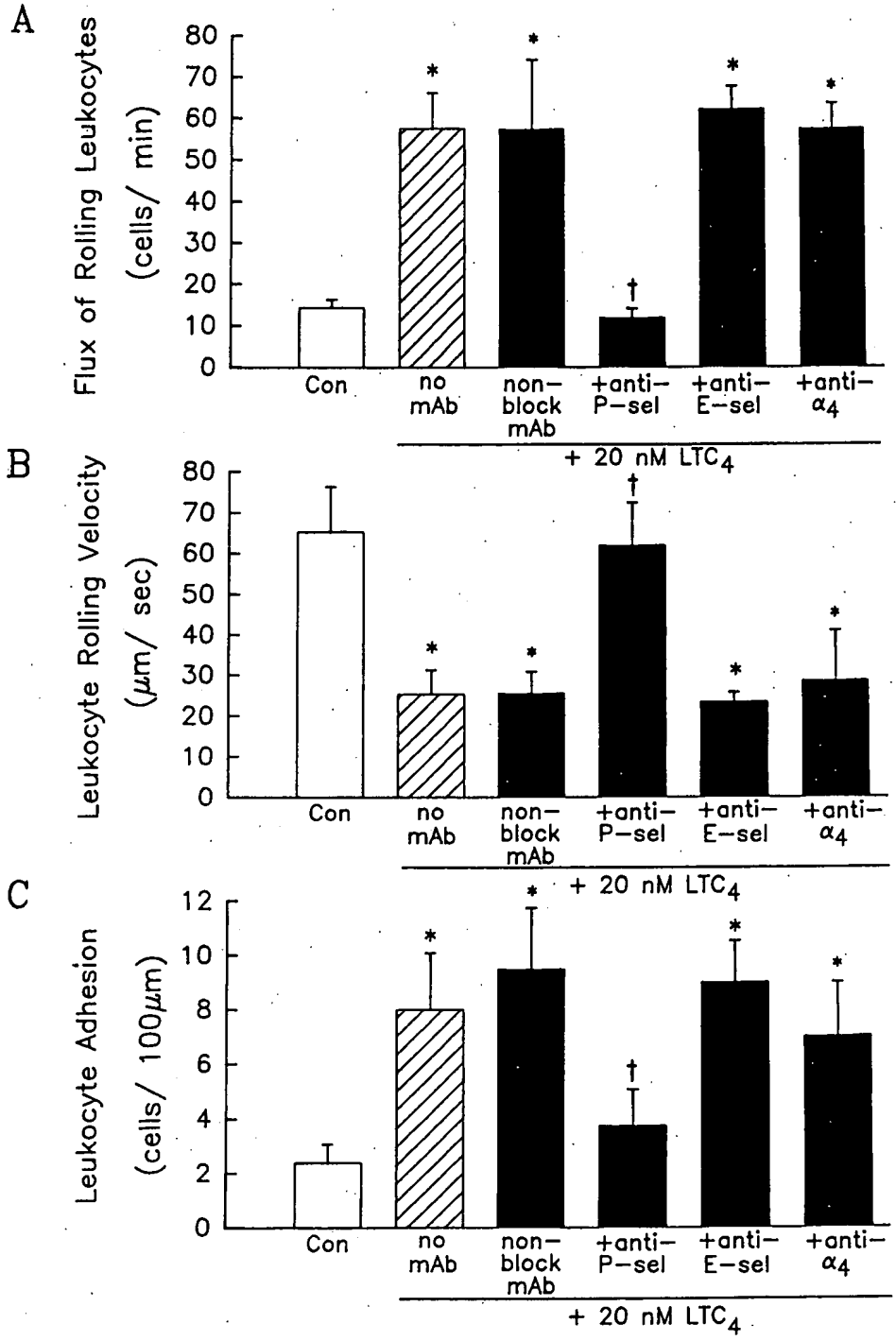


FIGURE 1. The effects of anti-P-selectin treatment on LTC_4-induced leukocyte recruitment. Leukocyte rolling flux (*A*), leukocyte rolling velocity (*B*), and leukocyte adhesion (*C*) in rat mesenteric postcapillary venules are shown. Vessels were superfused with 20 nM LTC_4 after an initial control period. A blocking anti-P-selectin mAb (2.5 mg/kg; RMP-1, $n = 5$) or a nonblocking anti-P-selectin mAb (2.5 mg/kg; RP-2, $n = 4$) was administered at 20 min. * $p < 0.05$ relative to time 0 min. † $p < 0.05$ relative to nonblocking mAb.

treatment and increased further between 90 and 150 min (Fig. 3*A*). Leukocyte adhesion was significantly increased by 30 min after initiation of treatment and continued to increase until 135 min, after which adhesion remained stable (Fig. 3*B*). Also noteworthy was the number of emigrated leukocytes observed in the extravascular tissues. After 90 min, leukocyte emigration was significantly elevated, and continued to increase over the experiment (Fig. 3*C*). Figure 3 also shows that there was no increase in leukocyte rolling flux or adhesion over 3 h in untreated animals, and only a subtle increase in emigration. In these experiments, leukocyte rolling velocity decreased by ~40% in LPS-treated animals (25.7 ± 4.9 μm/s at 180 min vs 41.4 ± 4.4 μm/s at 0 min; $p < 0.05$), but not in untreated animals (65.4 ± 12.4 μm/s at 180 min vs 54.5 ± 9.7 μm/s at 0 min).

In Figure 4 it can be seen that treatment with the blocking anti-P-selectin mAb (RMP-1) prevented the LPS-induced increases in leukocyte rolling flux and adhesion over the first 90 min of treatment (Fig. 4, *A* and *B*). However, leukocyte rolling and adhesion increased after this time point even though additional Ab was administered at 105 min. The P-selectin mAb



IGURE 2. Effects of anti-adhesion molecule therapy on LTC_4-induced leukocyte recruitment. Leukocyte rolling flux (*A*), leukocyte rolling elocity (*B*), and leukocyte adhesion (*C*) in rat mesenteric postcapillary venules are shown. Vessels were superfused with 20 nM LTC_4 after an itial control period. Abs against P-selectin (nonblocking RP-2, $n = 4$; or blocking RMP-1, $n = 5$), E-selectin (2.5 mg/kg; RME-1, $n = 4$), or the -integrin (4 mg/kg; TA-2, $n = 3$) were administered at 20 min. Values presented are the means of the 30-min time points and are representative f the Ab treatment effects. * $p < 0.05$ relative to untreated control. † $p < 0.05$ relative to 20 nM LTC_4.

so blocked LPS-induced leukocyte emigration (Fig. 4*C*), kely due to the reductions in leukocyte rolling and adhesion. ince leukocyte emigration was not normally observed until ter 90 min of LPS exposure, it is possible that the inhibition leukocyte rolling and adhesion by RMP-1 during the first 90 in delayed the onset of emigration by an additional 90 min 80 min total). Anti-P-selectin mAb also reversed the reduc- on in leukocyte rolling velocity, even at later time points (81.5 ± 8.6 μm/s at time 180 min), suggesting a P-selectin component even at 3 h.

Late LPS-induced leukocyte recruitment is dependent on L-selectin and the α_4-integrin

As P-selectin-independent leukocyte rolling and adhesion developed after 90 to 105 min of LPS superfusion, we characterized the recruitment mechanism(s) operating at these later time points. In

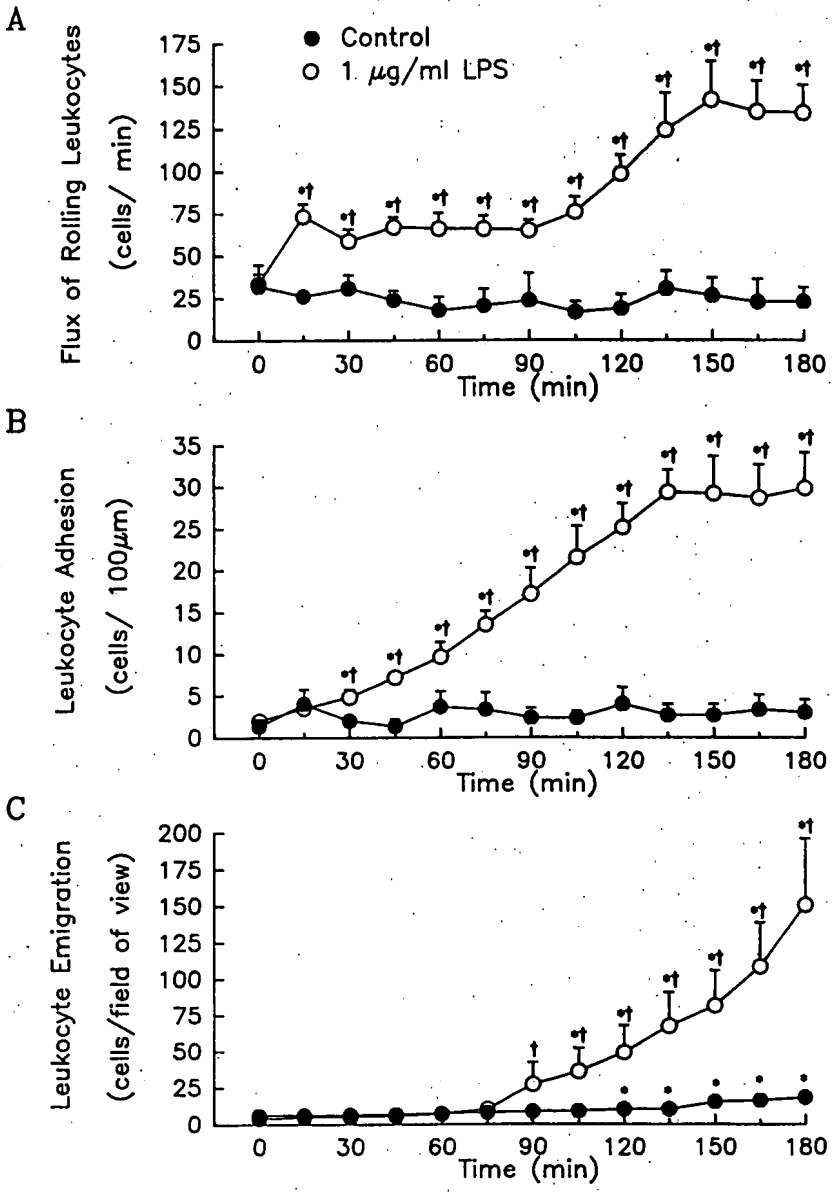


FIGURE 3. Local LPS administration induced leukocyte recruitment in rat postcapillary venules. Leukocyte rolling flux (*A*), leukocyte adhesion (*B*), and leukocyte emigration (*C*) were significantly increased by continuous superfusion of the mesentery with a solution containing 1 μg/ml LPS ($n = 6$). The mesentery of control rats ($n = 3$) was superfused with saline buffer. * $p < 0.05$ relative to time 0 min. † $p < 0.05$ relative to untreated control.

Figure 5 it can be seen that treatment with a blocking E-selectin mAb (RME-1) did not reduce leukocyte rolling flux or adhesion during the later phase of recruitment. However, treatment with anti-L-selectin Ab (HRL-3) or an anti-α_4-integrin Ab (TA-2) reduced leukocyte rolling and adhesion to baseline levels. This suggests that both molecules were utilized for the LPS-induced rolling at later time points. There were no further increases in leukocyte rolling velocity with any of these treatments (data not shown).

Leukocyte recruitment in adjuvant-induced vasculitis is dependent on L-selectin and the α_4-integrin

Figure 6 summarizes the role of the selectins in *M. butyricum*-treated animals at 12 days after immunization. The inset illustrates the Ab administration protocol. Function-blocking Ab was administered at 20 min, after two baseline observations (time 0 and 15 min), and the effects were followed over the next 45 min. The values in Figure 6*A* are the average leukocyte rolling flux observed over the 45 min after Ab treatment. Blocking Ab against P-selectin (RMP-1) did not reduce leukocyte rolling flux (Fig. 6*A* and inset) or adhesion (Fig. 6*B*). Leukocyte rolling flux was unaffected even if RMP-1 was given at three times the optimal concentration (7.5 mg/kg vs 2.5 mg/kg) (data not shown). Combined treatment with Abs against both P-selectin (RMP-1) and E-selectin (RME-1) also failed to reduce leukocyte rolling flux or adhesion (Fig. 6). Treatment with the combination of Abs to P-selectin (RMP-1), E-selectin (RME-1), and L-selectin (HRL-3) reduced leukocyte rolling flux by ~50%, implicating a role for L-selectin in leukocyte rolling 12 days after immunization (Fig. 6*A*). This combined anti-selectin treatment did not reduce leukocyte adhesion (Fig. 6*B*). None of the anti-selectin treatments caused significant changes in leukocyte rolling velocity (data not shown).

To confirm that the role for L-selectin in mediating leukocyte rolling was not due to overlapping adhesive mechanisms involving the other selectins, experiments were repeated using L-selectin Ab alone. In Figure 7*A* it can be seen that L-selectin Ab (HRL-3) reduced leukocyte rolling by ~55%, suggesting that L-selectin functions independent from the other selectins in this model. To characterize the mechanism mediating the remaining leukocyte rolling, animals were treated with a function-blocking Ab against the α_4-integrin (TA-2). Treatment with the α_4-integrin Ab on its own reduced leukocyte rolling by ~60% (Fig. 7*A*), indicating an important role for the α_4-integrin in mediating leukocyte rolling at

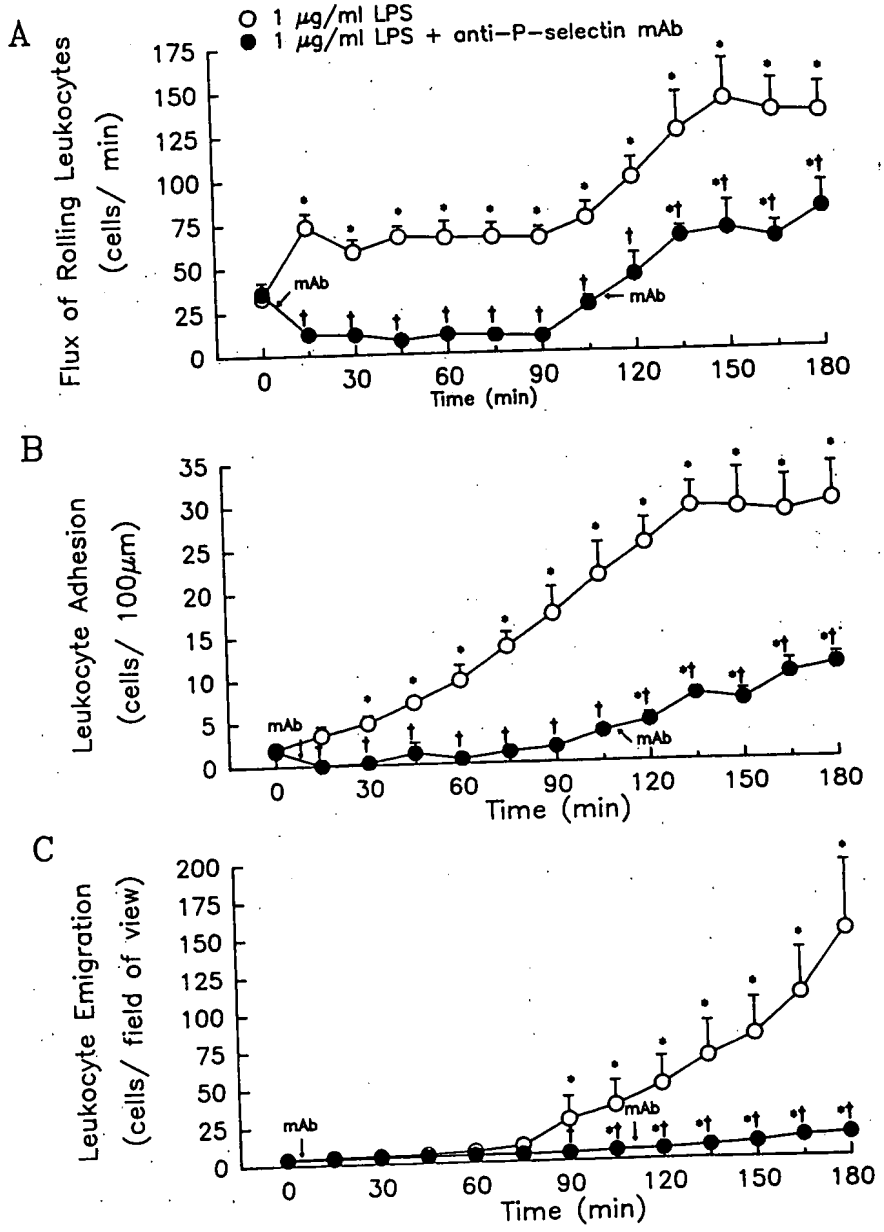


FIGURE 4. The effects of anti-P-selectin therapy on LPS-induced leukocyte recruitment. LPS-induced increases in leukocyte rolling flux (*A*), leukocyte adhesion (*B*), and leukocyte emigration (*C*) were significantly attenuated by an anti-P-selectin mAb (2.5 mg/kg; RMP-1, $n = 4$). The mesentery was superfused with a 1 μg/ml LPS solution throughout the experiment. * $p < 0.05$ relative to time 0 min. † $p < 0.05$ relative to LPS treatment alone.

day 12 following immunization. This treatment also reduced leukocyte firm adhesion by 75 to 80% (Fig. 7*B*), implicating roles for this molecule in both leukocyte rolling and firm adhesion. Administration of the anti-α_4-integrin Ab also increased the leukocyte rolling velocity by 47% (65.0 ± 3.9 μm/s vs 44.2 ± 3.3 μm/s untreated day 12, $p < 0.05$). Co-administration of anti-L-selectin and anti-α_4-integrin Abs reduced leukocyte rolling flux by ~90% (Fig. 7*A*). As this treatment was more effective than either Ab alone, an independent component for each adhesion molecule is suggested. Nevertheless, a flux of approximately 50 cells/min continued to roll. The combined treatment did not cause a further reduction in leukocyte adhesion (Fig. 7*B*), suggesting a primary role for the α_4-integrin in this interaction. This combination of Abs failed to increase leukocyte rolling velocity over the increase induced by the α_4-integrin mAb alone (60.2 ± 6.6 μm/s). In additional experiments, treatment with Abs against P-selectin, E-selectin, L-selectin, and the α_4-integrin did not reduce leukocyte rolling further (data not shown).

None of the Ab treatments had significant effects on circulating leukocyte counts in any of the models studied (Table I).

Discussion

Many different adhesion molecules have been implicated in the recruitment of leukocytes to sites of inflammation. However, this information has been obtained using a wide variety of techniques, reagents, and inflammatory models, making it difficult to directly compare recruitment mechanisms operating under different inflammatory conditions. This study is the first to systematically characterize the role of adhesion molecules in leukocyte recruitment to the same tissue under acute, subacute, and chronic inflammatory conditions.

In acute LTC_4-induced inflammation, increased leukocyte rolling was completely blocked by RMP-1, a new mAb against rat P-selectin. This inhibition of LTC_4-induced leukocyte rolling is consistent with our previous observations using a different mAb (PB1.3) raised against human P-selectin (15). However, neither RMP-1 nor PB1.3 were able to inhibit leukocyte rolling below baseline levels (~10–20 cells/min). Interestingly, RMP-1, which binds to a functional epitope on both rat and mouse P-selectin (17), as well as other anti-mouse P-selectin Abs eliminate leukocyte

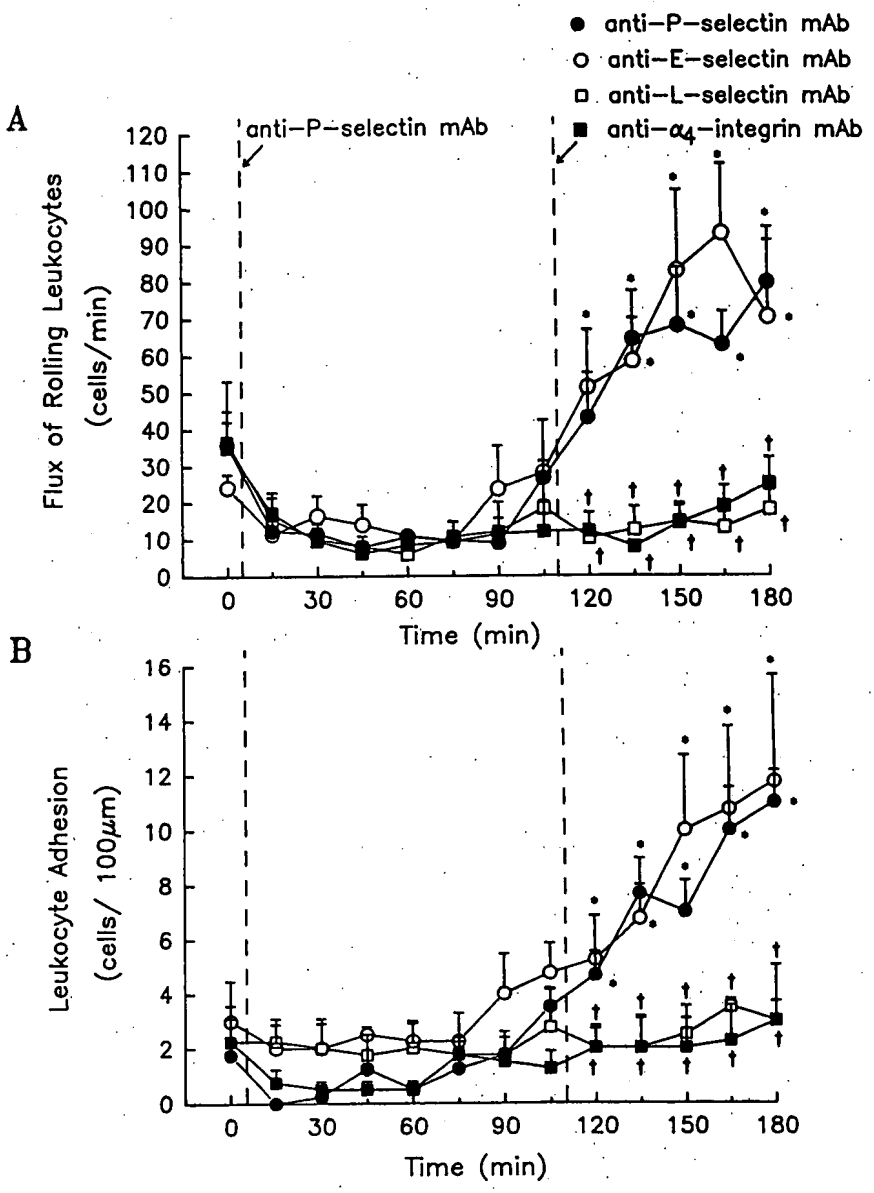


FIGURE 5. P-selectin-independent leukocyte recruitment mechanisms in the late phase of local LPS administration. Leukocyte rolling flux (*A*) and leukocyte adhesion (*B*) in rat mesenteric postcapillary venules are shown. Animals received anti-P-selectin mAb (2.5 mg/kg; RMP-1) at 5 min. A 1-μg/ml LPS solution was superfused over the mesentery throughout the experiment. At 105 min, rats were administered an additional dose of RMP-1 plus Ab against: E-selectin (2.5 mg/kg; RME-1, $n = 4$), L-selectin (1 mg/kg; HRL-3, $n = 4$), or the α_4-integrin (4 mg/kg; TA-2, $n = 4$). * $p < 0.05$ relative to time 0 min. † $p < 0.05$ relative to LPS + RMP-1 treatment.

rolling in mouse venules (Ref. 21, and our unpublished observations), raising the possibility that an additional rolling mechanism may exist in the rat. We have previously determined that baseline rolling is not dependent on L-selectin or the α_4-integrin (12), while the time course of this model is insufficient for the transcriptional up-regulation of E-selectin (22–26). These data all suggest that an alternate and as yet unidentified adhesive mechanism mediates baseline leukocyte rolling in rat mesenteric venules. Although the identity of this molecule remains unknown, it appears to have selectin-like functions as baseline rolling can be eliminated by the selectin blocking carbohydrate fucoidan (12). A similar mechanism may also exist in the feline mesentery (27).

In contrast to mAb PB1.3, RMP-1 was able to reverse the decrease in leukocyte rolling velocity caused by LTC_4. A possible explanation for this may be related to differences in Ab binding. RMP-1 binds to a conformational epitope in the lectin and/or epidermal growth factor domains of rat P-selectin (17), while PB1.3 binds in the complement repeats of P-selectin, a region not required for ligand binding (28). Rather than directly blocking a functional P-selectin epitope, PB1.3 may block rolling by steric hindrance or reducing the flexibility of P-selectin. In the presence of PB1.3, the lectin domain may still be available to facilitate slow rolling of leukocytes when rolling is initiated via an alternate mechanism.

In our model of LPS-induced inflammation, the early (acute) increase in leukocyte recruitment was also completely dependent on P-selectin. RMP-1 blocked leukocyte rolling over the first 90 min, after which there was an increase in leukocyte recruitment via P-selectin-independent mechanisms. This latter phase of leukocyte recruitment could be inhibited by Abs against either L-selectin or the α_4-integrin. These data suggest a sequential pattern in which these cells may use L-selectin to tether to the endothelium and then roll via the α_4-integrin. L-selectin has been demonstrated to mediate the initial attachment of leukocytes to the endothelium in vitro and in vivo (12, 29), and the absence of α_4-dependent leukocyte rolling in the presence of L-selectin Ab supports this sequential pattern.

The overlapping requirements for L-selectin and the α_4-integrin are consistent with the adhesion cascade reported under shear conditions for eosinophils and monocytes (10, 30, 31). L-selectin appears to mediate the initial tethering of these cells to the endothelium while the α_4-integrin mediates the transition from rolling to

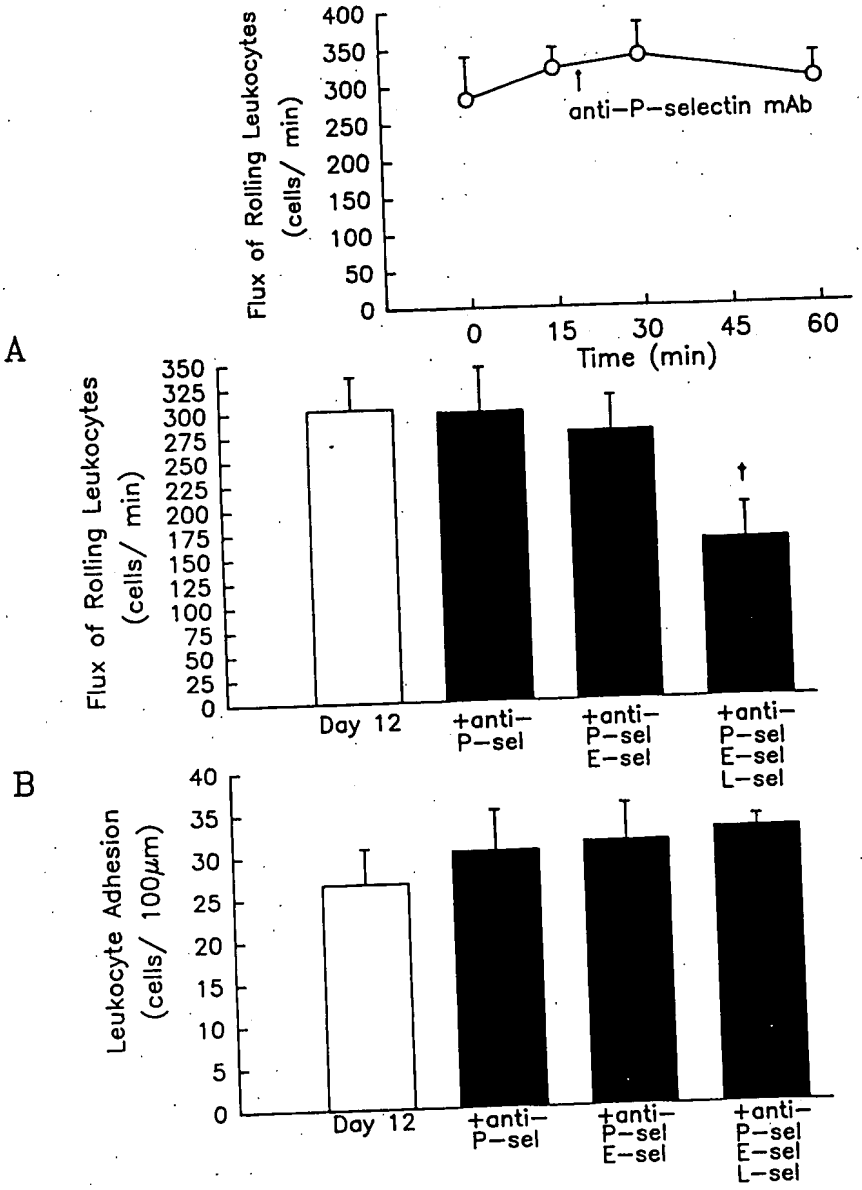


FIGURE 6. Effect of anti-selectin therapy on leukocyte rolling flux (*A*) and leukocyte adhesion (*B*) in mesenteric venules of *M. butyricum*-immunized (day 12) animals. Immunized animals were treated with combinations of mAbs against: P-selectin (2.5 mg/kg; RMP-1, $n = 4$), P-selectin + E-selectin (2.5 mg/kg; RME-1, $n = 4$), or P-selectin + E-selectin + L-selectin mAb (1 mg/kg; HRL-3, $n = 4$) at 20 min into a 60-min protocol. This administration protocol is illustrated in the inset. The administration of RMP-1 at 20 min did not affect leukocyte rolling flux. The time points after Ab administration were compared with the initial baseline time points within each animal. † $p < 0.05$ relative to untreated day 12 immunized animals.

firm adhesion. Although rat neutrophils have been shown to express the α_4-integrin (32), this molecule does not appear to mediate baseline (12) or LTC_4-induced leukocyte rolling (this study). It is not known whether the α_4-integrin can be up-regulated to mediate neutrophil recruitment at the later stages of LPS administration, and it remains to be determined what cell types are interacting with the endothelium at this time point.

The role of E-selectin in inflammation is not entirely clear. E-selectin expression is induced on the surface of cultured endothelium 2 to 4 h after treatment with LPS, TNF-α, or IL-1 (22, 26, 33). However, the anti-rat E-selectin mAb (RME-1) was unable to block leukocyte recruitment in rat mesenteric venules treated with LPS, despite its ability to block binding to E-selectin in vitro (18), and reduce leukocyte recruitment to inflamed joints and sites of dermal inflammation in vivo (34). Previous Ab studies using reagents and cells that cross species have produced equivocal results with respect to E-selectin. An anti-human E-selectin Ab reduced the rolling of isolated human neutrophils injected into IL-1-stimulated rabbit mesenteric venules (25, 35), whereas a different anti-human E-selectin Ab blocked feline neutrophils from rolling on stimulated feline endothelium in vitro but could not block TNF-α-induced rolling in cat mesenteric venules (36). The present study is the first to use a rat E-selectin Ab to examine leukocyte recruitment in the rat mesentery using a stimulus known to induce E-selectin expression in vitro. Our data support the notion that E-selectin is not important in this vascular bed. It appears that E-selectin may be more selective for leukocyte recruitment to other tissues, as E-selectin has been implicated in leukocyte recruitment to dermal inflammation in the pig (37) and rat (34), and LPS-induced leukocyte recruitment to the murine lung (38).

We have previously reported roles for the α_4-integrin (~50%), P-selectin (~20%), and L-selectin (~50%) in leukocyte recruitment 4 days after immunization with *M. butyricum* (12). Twelve days after immunization, the recruitment pattern appears to exclude a role for P-selectin and no role could be found for E-selectin, even when other adhesion molecules were blocked. However, 90% of leukocyte rolling could be eliminated by the tandem administration of Abs against L-selectin and the α_4-integrin. Unlike the LPS model, these treatments were additive rather than overlapping, suggesting independent adhesive cascades. It is possible

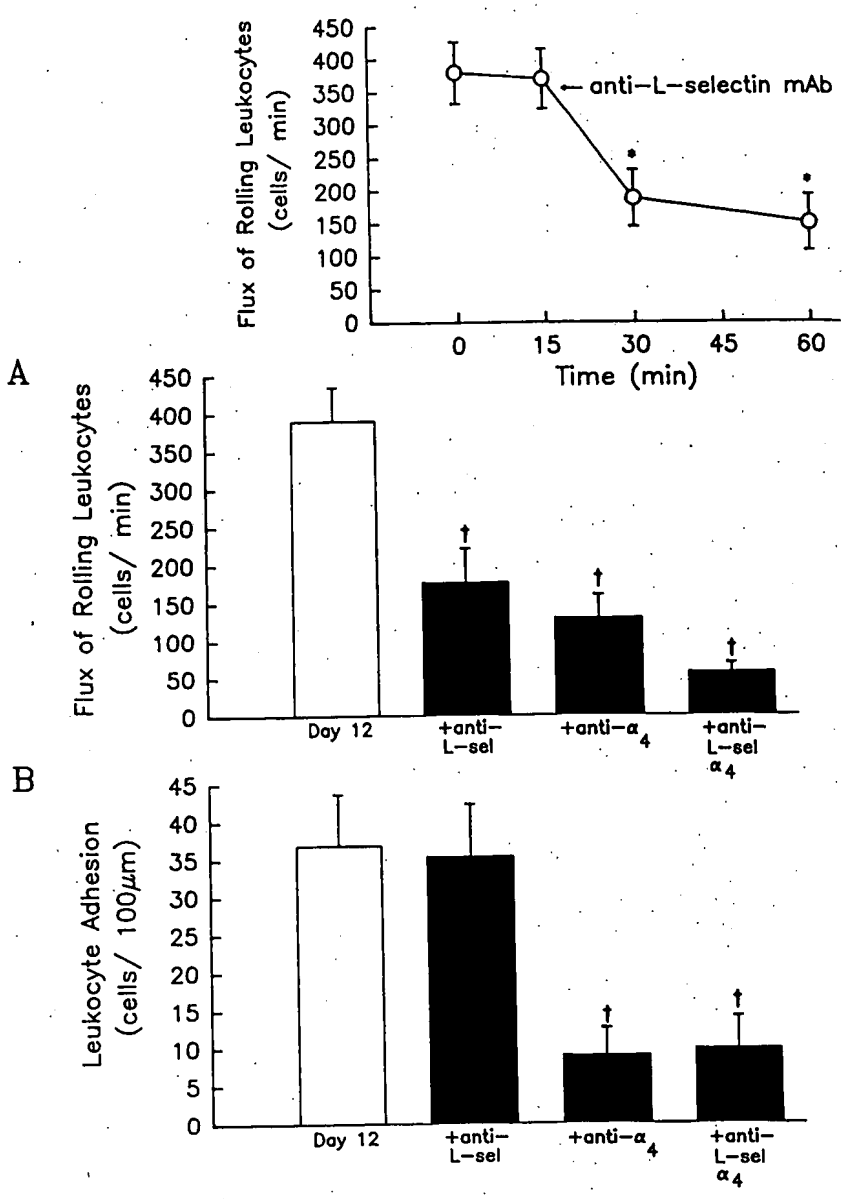


FIGURE 7. Effect of anti-L-selectin and α_4-integrin therapy on leukocyte rolling flux (*A*) and leukocyte adhesion (*B*) in mesenteric venules of *M. butyricum*-immunized (day 12) animals. Immunized animals were treated with an anti-L-selectin mAb (1 mg/kg; HRL-3, $n = 4$), an anti-α_4 mAb (4 mg/kg; TA-2, $n = 4$), or both ($n = 4$) 20 min into a 60-min protocol. The administration protocol is illustrated in the inset. The administration of HRL-3 at 20 min significantly reduced leukocyte rolling flux at 30 and 60 min. The time points after Ab administration were compared with the initial baseline time points within each animal. * $p < 0.05$ relative to time 0 min. † $p < 0.05$ relative to untreated day 12 immunized animals.

Table I. *Circulating leukocyte counts in animals receiving mAb treatments*

Treatment	Leukocyte Counts ($\times 10^5$ cells/ml)					
	No mAb	RP-2	RMP-1	RME-1	HRL-3	TA-2
LTC_4	77.3 ± 9.2	71.7 ± 12.8	82.2 ± 9.9	61.0 ± 3.4	ND	101.64 ± 17.4
LPS[a]	74.0 ± 7.6	ND	78.6 ± 5.2	67.0 ± 3.8	77.4 ± 5.9	70.3 ± 4.5
M. butyricum[b]	169.7 ± 11.9*	ND	187.2 ± 7.7*	189.3 ± 7.8*	222.9 ± 13.9*	211.5 ± 19.3*

[a] LPS-treated animals given RME-1, HRL-3, or TA-2 also received RMP-1.
[b] *M. butyricum*-immunized animals given RME-1 also received RMP-1.
* $p < 0.05$ compared with LTC_4 and LPS-treated animals. Antibody treatments did not cause significant changes in circulating leukocyte counts.

that two cell populations may be recruited in this model, one rolling via L-selectin and the other rolling via the α_4-integrin. However, the dual administration of Abs against the α_4-integrin and L-selectin did not completely inhibit leukocyte rolling. A significant number of cells continued to roll in mesenteric venules of *M. butyricum*-immunized rats even after treatment with Abs against P-, E-, L-selectin and the α_4-integrin (~50 cells/min), suggesting an additional adhesion molecule may be present. The existence of another adhesion molecule able to mediate leukocyte tethering and rolling is consistent with an in vitro report of a novel selectin-like adhesion molecule up-regulated on the surface of cultured endothelium 24 h after IL-1 stimulation (39). This ligand mediates leukocyte rolling via carbohydrate moieties presented by L-selectin and other neuraminidase-sensitive ligands expressed on the leukocyte (39).

This paper demonstrates that different leukocyte adhesive mechanisms are evoked in acute, subacute, and chronic inflammatory settings. The novel findings of this study include roles for L-selectin and the α_4-integrin in LPS- and adjuvant-induced leukocyte recruitment. Additionally, we could not find a role for E-selectin in

acute, subacute, or chronic mesenteric inflammation in the rat. Finally, a P-, E-, and L-selectin (and α_4-integrin)-independent rolling pathway appears to exist in chronically inflamed microvessels in the rat. Future work is directed toward identifying the type of rolling and adhering leukocytes in vivo, as differences in recruited cell type(s) likely underlie the different adhesive pathways observed during acute, subacute, and chronic inflammatory processes. Clearly, the adhesive cascade that mediates leukocyte recruitment can change in different inflammatory settings depending on the stimulus, the time course, and the tissue that is affected. Therefore, potential anti-inflammatory strategies devised to block leukocyte recruitment in disease states must be designed carefully to target the adhesive mechanisms that are important in that inflammatory setting.

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